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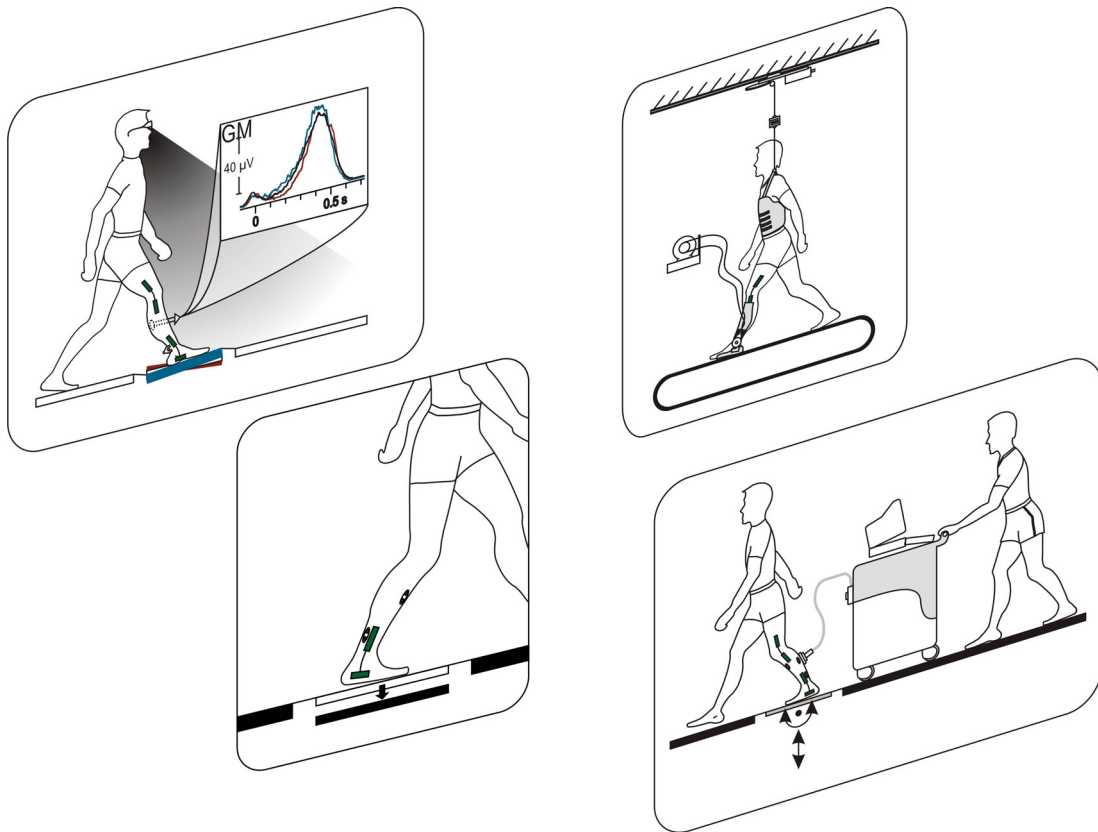
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Force related afferent feedback to the triceps surae locomotor activity during human walking

by

Richard af Klint



Dissertation submitted for the degree of Doctor of Philosophy
Center for Sensory-Motor Interaction
Aalborg University

Aalborg 2009

Preface

This Ph.D. dissertation, entitled "Force related afferent feedback to the triceps surae locomotor activity during human walking", is based on four original papers that were published or submitted to international peer-reviewed journals. The studies are referred throughout this dissertation by roman numerals **I-IV**. The work reported in **I-IV** was carried out between September 2005 to December 2008 at the Center for Sensory-Motor Interaction at Aalborg University, Aalborg, Denmark.

- I Within-step modulation of leg muscle activity by afferent feedback in human walking.** af Klint R, Nielsen JB, Cole J, Sinkjaer T, Grey MJ. J Physiol. 2008 Oct 1; 586(Pt 19):4643-8. doi: 10.1113/jphysiol.2008.155002
- II Sudden drop in ground support produces force related unload response in human over-ground walking.** af Klint R, Nielsen JB, Sinkjaer T, Grey MJ. Neurophysiol. 2009 Jan 21. doi: 10.1152/jn.91175.2008
- III Load rather than length sensitive feedback contribute to soleus muscle activity during human treadmill walking.** af Klint R, Mazzaro N, Nielsen JB, Sinkjaer T, Grey MJ; submitted to J. Neurophysiol. 2009
- IV Afferent contribution to locomotor muscle activity during unconstrained over-ground human walking: an analysis of triceps surae muscle fascicles.** af Klint R, Cronin NJ, Ishikawa M, Sinkjaer T, Grey MJ; submitted to J. Neurophysiol. 2009

Cover image adapted from figure 1 from **I-IV**

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Declaration

I hereby declare that this is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person, nor material which to a substantial extent has been accepted for the award of any other degree or diploma at Aalborg University or other institute of higher learning, except where due acknowledgement has been made in the text.

I also declare that the intellectual content of this dissertation is the product of my own work, except to the extent that assistance from others in the project's design and conception or in style, presentation and linguistic expression is acknowledged.

Richard af Klint

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- My dear colleagues at SMI – making these years a memorable experience. Special thanks to my roommates Jonas Emborg & Strahinja Došen for inspiring conversations on everything from baby food to afferent firing rates.
- My wife and daughter – for their unconditional love and support. Special thanks to Camilla for making this journey with me and moving to exotic Denmark far away from friends and family.
- My parents and siblings – for their continuing support and their interest in my academic pursuits.

Abbreviations

ATF	Achilles tendon force
BWS	body weight support
CPG	central pattern generator
EMG	electromyogram
H-reflex	Hoffmann reflex
GM	gastrocnemius medialis
GTO	Golgi tendon organ
MLR	medium latency response
SLR	short latency response
SOL	soleus
TA	tibialis anterior

English summary

Human walking is in essence a balancing act. The leg muscles' activation is in constant need of adaptation to keep a stable and efficient gait. In this Ph.D. dissertation I investigated if our bodily sensors are used in the tuning of the leg muscles' activity and whether the same sensors also activate the muscles through a feedback loop in the spinal cord. More specifically, the first objective was to investigate which proprioceptive afferents contribute to the activation of the soleus muscle in human walking. This was addressed by two experimental setups that unload the plantar flexor muscles during stance. A movable platform was used to perturb the foot during unconstrained over-ground walking by accelerating downwards, and thereby reducing the ground reaction force. It was hypothesised that the decreased force on the ankle extensors would result in a reduction of afferent feedback on the soleus muscle. A short lasting but significant decrease in locomotor activity was found. To investigate which afferents contributed to this decreased activity the muscle length was controlled and also muscle fascicle lengths were monitored during the acceleration of the platform. Both investigations showed length and lengthening velocity to be significantly delayed to the perturbation indicating that most likely the decrease in soleus activity is the result of force feedback from Golgi tendon organs originating in the Achilles tendon.

The second setup combined transient body weight support (BWS) to change the load on the supporting leg and rapid plantar flexion perturbations during stance. The potential load feedback was quantified by the change in depression of the locomotor activity induced by unloading the plantar flexor muscles as the ankle was extended. The hypothesis was that if load feedback contributes to the generation of locomotor activity the depression would decrease with the decreased load. The unload response was reduced as hypothesised, and furthermore, the unload response was not significantly reduced by depression of the group II afferent pathway. As previous studies have indicated that neither group Ia nor cutaneous afferents are essential for this response, the current result supports the aforementioned hypothesis that force sensitive afferent contributes to the locomotor activity.

The second objective was to investigate whether proprioceptive afferent feedback is modulating the locomotor output in over-ground human walking. A robotic platform mounted flush in the floor of the laboratory perturbed the foot during the stance phase of unconstrained walking. Prior to subjects' heel contact, the platform rotated to one of five inclinations ($<\pm 3^\circ$) in the parasagittal plane and held this rotation throughout stance. It was hypothesised that the slight changes in inclination would change afferent firing rates, and if these afferents project on the motoneuron the plantar flexor activity would change with respect to these changes in activity. The triceps surae activity was increased for the positive inclinations and decreased for the negative inclinations. A negative or no changes

were found for the muscle-tendon lengthening velocity and muscle fascicle velocity changes, respectively. However, the activation changes were correlated to changes in Achilles tendon force estimates and muscle-tendon and muscle fascicle length. This indicates that plantar flexor activity is influenced by afferent activity sensitive to force (group Ib) and/or length (group II) changes. Furthermore a deafferented subject was recruited for this experiment with the hypothesis that if the effect seen in healthy is the result of vestibular or visual feedback, a subject that has heightened sensitivity to vestibular perturbations and solely uses visual feedback for muscle control would have an increased response. This was not the case. No difference between baseline and a positive inclination was found, indicating that the effect seen in healthy subjects is mediated through afferent feedback.

In summary, the results from the current investigations suggest that proprioceptive feedback enhances the centrally mediated locomotor activity. It is likely that proprioceptive enhancement is, at least in part, mediated by feedback of group Ib force sensitive afferents. Furthermore, proprioceptive afferents adapt the locomotor output in a functional way during the stance phase. This adaptation to very small changes in the support surface is likely enabled through positive feedback of force sensitive group Ib and/or length sensitive group II afferent drive. Hence, proprioceptive feedback can enhance and adapt the locomotor output to fit the walking environment thereby constituting the first line of control in the complex control of human walking.

Danish summery

Menneskets gang er i bund og grund en balanceakt. Aktiveringen af benets muskler har konstant brug for tilpasning for at opretholde en stabil og effektiv gang. I denne PhD-afhandling har jeg undersøgt, om menneskets kropslige sensorer bruges til at afstemme aktiviteterne i benets muskler, og om de samme sensorer også aktiverer musklerne gennem en feedback-sløjfe i rygmarven. Mere specifikt var det første mål at undersøge menneskets gang, og herunder hvilke proprioceptive afferenter, der bidrager til aktiveringen af soleus musklen. Denne undersøgelse blev baseret på to forsøg, som aflastede plantar flexor musklerne under standfasen. En bevægelig platform blev anvendt til at påvirke foden under uhindret gang ved hjælp af en acceleration i nedadgående retning med en deraf følgende reduktion af den kraft, som opstår mellem foden og underlaget (ground reaction force). Hypotesen var, at den reducerede kraft på ankel-ekstensorerne ville betyde en reduktion af den afferente feedback på soleus muskelen. Der blev påvist en kortvarig - men betydelig - reduktion i den bevægelsesmæssige aktivitet. For at undersøge hvilke afferente nerver, der bidrog til denne reduktion i aktivitet, blev muskel-længden kontrolleret. Ligeledes blev længden af muskelbundterne overvåget under platformens bevægelse. Begge undersøgelser viste, at længden og forlængelseshastigheden blev væsentligt forsinket ved perturbationen, hvilket indikerer at reduktionen i soleus-aktivitet sandsynligvis skyldes kraft-feedback fra Golgi sene-organet i akillessenen.

Det andet forsøg kombinerede transient kropsvægt-støtte (BWS) til ændring af belastningen af det støttende ben med hurtige plantarfleksions forstyrrelser under standfasen. Den potentielle kraft-feedback blev kvantificeret som ændringen i undertrykkelsen af bevægelsesmæssige aktivitet forårsaget af aflastningen af plantar flexor musklerne, når ankelen blev strakt. Hypotesen var, at hvis belastnings-feedback bidrager til fremkaldelsen af bevægelsesmæssige aktivitet, ville undertrykkelsen minske med den minskede belastning. Aflastningsresponsen blev reduceret som antaget, og endvidere blev aflastningsresponsen ikke væsentlig reduceret af undertrykkelse af Gruppe II afferente baner. Da tidligere studier har vist, at hverken Gruppe Ia eller kutane afferente nerver er væsentlige for denne respons, støtter de foreliggende resultater ovennævnte hypotese om, at kraft-følsomme afferente nerver bidrager til den bevægelsesmæssige aktivitet.

Det andet formål var at undersøge, om den proprioceptive afferente feedback modulerer den bevægelsesmæssige aktivitet under menneskets gang. En bevægelig platform, der var monteret flugtende med gulvet i laboratoriet, påvirkede foden i stand-fasen under uhindrede gang. Umiddelbart før forsøgspersonens hæl fik kontakt med underlaget, roterede platformen til én ud af fem mulige hældninger ($\pm 3^\circ$) i det parasagittalt plan, og denne rotation blev fastholdt i hele stands-fasen. Det blev antaget, at de små ændringer i hældningen ville påvirke den afferente aktivitet, og hvis de afferente nerver projicerer på motoneuron, ville plantar-fleksor aktiviteten ændre sig i takt med disse ændringer i aktiviteten. Aktiviteten i

triceps surae musklen blev forøget ved de positive hældninger og reduceret ved de negative hældninger. Der blev fundet en negative eller ingen ændringer for henholdsvis forlængelseshastighed for muskel-sener og for hastighedsændringer i muskel-bundter. Dog, var ændringen i aktivitet korreleret til ændringer i estimeret af akillessenens kraftbelastning samt længden af muskel-senen og muskel-bundter. Dette indikerer, at plantar-fleksor aktivitet påvirkes af afferent aktivitet følsomme overfor kraft (gruppe Ib) og / eller længde (gruppe II) variationer. Endvidere deltog en forsøgsperson der ikke havde afferent feedback (deafferented) i dette forsøg. Hypotesen var, at hvis virkningen, som ses i raske forsøgspersoner, er et resultat af vestibulær eller visuel feedback, vil en forsøgsperson, som har øget følsomhed over for vestibulære perturbationer, og som kun bruger visuel feedback til muskelkontrol, have en forøget respons. Dette var ikke tilfældet. Der blev ikke fundet forskelle mellem udgangspunktet og en positiv hældning, hvilket indikerer, at resultatet, som ses i raske forsøgspersoner, er medieret gennem afferenter.

Sammenfattet tyder resultaterne fra disse undersøgelser på, at proprioceptiv feedback forstærker den centralt formidlede bevægelsesmæssige aktivitet. Det er sandsynligt, at den proprioceptive forstærkning som minimum delvis er formidlet af feedback fra Gruppe Ib kraftfølsomme afferente nerver. Endvidere tilpasses det bevægelsesmæssige output sig på en funktionel måde under stand-fasen grundet proprioceptive afferenter. Denne tilpasning til meget små ændringer i støtteoverfladen bliver sandsynligvis muliggjort gennem et positiv feedback fra kraftfølsomt Gruppe Ib og/eller længde-følsomme Gruppe II afferente nerver. Således kan proprioceptiv feedback forstærke og tilpasse det bevægelsesmæssige output til gangmiljøet og derved udgøre den første del af den komplekse kontrol af menneskets gang.

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I Introduction

Human gait is very flexible; we inhabit and manoeuvre in vastly different environments, from the sandy hills in the Sahara desert to the mountain ridges in Switzerland. This flexibility comes at a drawback; human bipedal gait is a complex task setting more stringent demands on the control than for quadrupeds. Our bipedal gait is a balancing act of the falling body that is supported most of the time by a single leg. To control the human gait we are dependent on the constant adaptation of the muscle contractions to the surrounding environment without which falling would be inevitable. All the while this control is performed naturally and seemingly without any conscious involvement.

The intriguing network of neurons that enable this control is the focus of this PhD dissertation. However, I limit my studies to the shortest and fastest feedback loops as the complexity of the full system is outside the scope of any 3 year study. To put it into perspective, locomotion in animals and humans has been the interest of scientists ever since the ancient Greek. But only recently has technical advances enabled careful studies of the role of somatosensory feedback in the control of the leg muscles in animals. In cats as well as other vertebrates and non-vertebrates force/load feedback has been shown to play an important role in the control of locomotor activity (Duysens *et al.* 2000). For example, force sensing group Ib afferents, in series with the ankle extensor muscles, have an excitatory feedback drive to the motoneurons during the stance phase in walking cats (Pearson and Collins 1993; Donelan *et al.* 2009). The ankle extensor muscles share the role of generating the body's forward propulsion with the hip and knee in the cat (Nielsen 2003). Hence, the positive force feedback loop could automatically adjust muscle activity to compensate for higher loads faced as the animal walks uphill. In human walking, the ankle extensor activity in late stance phase is even more crucial for the propulsive forces (Nielsen 2003) making, at least for the late stance, feedback from force sensors a potentially relevant control input. However, when studying humans we are left with less invasive measuring techniques which have made deciphering the sensory feedback more complicated than for the animal models. This dissertation has the aim to address some of the unanswered questions regarding how sensory afferent feedback in human walking controls the lower leg muscle activity.

2 Background

Human walking has evolved over time and our skeletal, muscular and tendinous tissue as well as our nervous system has adapted to this form of locomotion (Nielsen 2003). Though much is known about the kinetic and kinematics of walking we have so far only brushed at the surface of the complexity of neural control underlying our automated behaviour. The neural circuitry of walking integrates control signals at cortical and spinal level, adjusts the motor pattern based on sensory information from visual, vestibular and somatosensory system. Furthermore, the neural control needs to control a multi-segmental body with vast degrees of freedom using muscles that all have different dynamics and mechanical coupling to the segments.

The general consensus is that there are several layers of control mechanisms at play in order to control of walking: descending control signals from supraspinal structures (Armstrong 1988), the activation of rhythmic neural networks within the spinal cord (central pattern generators, CPG, Grillner 1975), and the integration of somatosensory information from the periphery (for review see Zehr and Stein 1999; Duysens *et al.* 2000; Rossignol *et al.* 2006). The CPG has been carefully mapped in the lamprey (Grillner 1975) and its existence in lower vertebrates and cats spinal cord is beyond doubt (Rossignol *et al.* 1996). However, the existence of a CPG in humans is still elusive. The indirect evidence of its existence comes from spinal cord injured (Calancie *et al.* 1994; Dimitrijevic *et al.* 1998) and infants (Yang *et al.* 1998). Following complete spinal cord transection, locomotion is seen in cats after training, but not in humans. This difference could be due to the demands on bipedal walking and the benefit of disassociate movements of the upper and lower extremities has put CPG under stronger supraspinal control in the healthy adult human. Similarly, in non-human primates the CPG appears to be dependent on supraspinal activation to function normally (Eidelberg *et al.* 1981; Fedirchuk *et al.* 1998). In any case, the fact that locomotor activity is not readily seen in spinal cord injured man and the inherent constraint to indirect measures has made the CPG's existence difficult to conclusively prove in the human.

Sensory feedback from the vestibular organs, from the visual system, and from the somatosensory organs is important for the control of walking. The somatosensory information is composed of the sense of touch, pain and proprioception. Proprioception refers to the sense of the body's own movements. In this dissertation I have adopted the broad view of proprioception from Prochazka (1996), including all mechanoreceptors that potentially contribute to the sense of the body's position and orientation in space and its internal forces, whether the receptor output contributes to the conscious sense or not. The sense of the body comes from the muscle spindles sensing the length and lengthening velocity of the muscle fibres, the Golgi tendon organs sensing the force on the muscle, the joint receptors mostly sensitive at the extreme of the joint's range of motion and

stretch sensitive skin receptors. Proprioceptive feedback has been implicated in switching between phases (Duysens and Pearson 1980), enabling rapid corrective response to mechanical perturbations (Dietz *et al.* 1984; Yang *et al.* 1991; Sinkjaer *et al.* 1996), contributing to the locomotor activity (Sinkjaer *et al.* 2000; Grey *et al.* 2004; Grey *et al.* 2007), and modulating the locomotor activity (Mazzaro *et al.* 2006; Stephens and Yang 1999; Bachmann *et al.* 2008).

The importance of proprioceptive feedback is apparent when the proprioceptive signals are blocked. The removal of sensory information has been extensively studied in animal models using a procedure called deafferentation. This procedure involves a transection of the sensory dorsal roots blocking sensory nerve traffic to the CNS while leaving the motoneuron pathways intact (e.g. Grillner 1975). Deafferentation can also be achieved through vitamin B6 overdose which destroys the large afferents (e.g. Pearson *et al.* 2003). Also in humans “deafferentation” has been studied in subjects suffering from rare forms of selective sensory neuropathy (e.g. Sanes *et al.* 1984; Ingram *et al.* 2000). In these subjects autoimmune responses to viral infections cause the myelinated sensory afferents to deteriorate and stop functioning. To fill the gap in their muscle control, visual input is consciously used and without it they are left with rudimentary and uncontrollable muscle contractions as they have no information about the orientation of their bodies. These subjects could potentially use purely feed-forward control of their muscles, but even sustaining a static posture requires the feedback from sensory signals (Sanes *et al.* 1984). Even the slightest of perturbations will need to be counteracted during bipedal walking and standing as our body is a very unstable system. Think of it as balancing a stick in your hand; without the adjustments of your hand, a light puff of air will make the stick fall to the ground. Therefore, it is beyond doubt that somatosensory and especially proprioceptive feedback is crucial for the normal and virtually unconscious control of human walking.

Throughout this dissertation two terms are used when discussing the implication of afferent feedback: contribute and modulate. This separation has its historical origin in the experimental paradigms used by Sinkjaer *et al.* (2000) and has relevance for how the results may be discussed. The Sinkjaer *et al.* (2000) study showed that the removal of afferent feedback resulted in a decrease in the level of locomotor activity. This change in locomotor activity could have several physiological explanations, e.g. the afferent drive could be gaiting locomotor programs or the afferent drive could through a feedback loop contribute in a continuous manner and thereby also modulate the locomotor output. Hence, in this dissertation the wording afferent contribution implies that the afferent activity raises the level of locomotor activity but does not imply that the afferent activity shapes the magnitude of the locomotor activity. Afferent modulation, on the other hand, is used to describe a process where the level of afferent activity influences the level of locomotor activity. Different experimental paradigms may be necessary to indicate either a modulating or a contributing function and hence the distinction.

2.1 *Afferents and sensors*

In this dissertation the feedback from the large proprioceptors and skin afferents is discussed and therefore I have in more detail outlined the physiology of these sensors in the section below. The scope of this section is to give the reader an understanding of the output on a systems level rather than in detail characterise each sensor.

2.1.1 *Muscle spindles*

Muscle spindles are located amongst the muscle fibres in all but a few skeletal muscles. They consist of a four to eight intrafusal muscle fibres in a capsule of connective tissue. The spindles range in length from 0.5–10mm and lie in parallel with the extrafusal muscle fibres (Prochazka 1996). There are three types of intrafusal muscle fibres: one dynamic bag₁ (DB1 or b₁), one static bag₂ (SB2 or b₂) and 2–6 smaller chain fibres (Kandel *et al.* 2000). The larger bag fibres have their nuclei collected in an expanded region in the centre of the fibre while the chain fibres have their nuclei lined up single file and hence the naming convention. Primary group Ia and secondary group II myelinated sensory axons innervate the spindle by encircling the non-contractile central part of the intrafusal muscle fibres (Prochazka 1996).

The sensory endings of the Ia afferent fibre contact all intrafusal muscle fibres while the II afferent fibres contact the b₂ and chain fibres. The difference in properties of the intrafusal muscle fibres makes the group II afferents more selectively sensitive to length changes than the group Ia which is mostly velocity sensitive. Both the primary and secondary endings show linear responses to length changes. However, for the primary endings this linear region is very limited, below 0.1mm at 1Hz sinusoidal stretches, after which the sensitivity to length changes attenuates (Matthews and Stein 1969b). For the secondary endings this linear region extends much further, ≥ 2 mm at 1Hz sinusoidal stretches (Matthews and Stein 1969b), making the secondary endings normally within the linear range during physiological conditions. Furthermore, the primary and secondary endings response to velocity and length changes is regulated by the gamma motor system, a motor neural system activating the intrafusal muscle fibres. There are two classes of gamma-fusimotor axons, the static and the dynamic gamma fibres, activating intrafusal muscle fibres. This gamma activation of the intrafusal muscle has a nonlinear effect on the sensitivity of the primary and secondary endings (Brown *et al.* 1965; Matthews and Stein 1969a).

In the early 1950's Merton proposed that there exists a linkage between alpha and gamma activity during active contractions (Merton 1953). The theory was that the gamma system would regulate the spindle output in such a fashion that the feedback from spindle afferents would work as the error signal between the intended movement and the real movement. When contracting the muscle, the gamma motor neurons would activate the intrafusal muscle fibres that shorten to

the intended length. Any divergence from the intended shortening would be sensed by the spindle afferent firing. Alpha-gamma coactivation in cats is state dependent in order to potentially tune the sensitivity of the spindle afferents to the different needs of motor control (Prochazka *et al.* 1985). The majority of the evidence from human subjects do not support an independent gamma control, though precision tasks raise the fusimotor activity (Kakuda *et al.* 1996). However, recent evidence challenge this view (Ribot-Ciscar *et al.* 2009). When subjects were more focused on determining the velocity of a perturbation the dynamic response of the plantar flexor spindle afferents was increased, and vice versa for the static response when the subject were focused on the position of the perturbation. This indirectly shows a tuning of the gamma drive to the specific condition. Nevertheless, that the alpha-gamma coactivation may be tuned during different conditions does not remove its fundamental importance to regulate the input to spinal cord. However, the potential of task dependent tuning of gamma drive further indicates the need to investigate the afferent contribution to locomotor activity in a natural environment and while walking.

In humans, spindle afferent firing rates have been studied for both large-amplitude muscle stretches (Edin and Vallbo 1990a; Edin and Vallbo 1990b; Edin and Vallbo 1988) and low-velocity, small-amplitude stretches(Kakuda 2000). Kakuda (2000) measured the spindle responses for a 1Hz sinusoidal stretch with amplitudes ranging from approximately 0.03-0.96% of rest lengths in relaxed muscle. The response of primary and secondary afferents was linear up to 1° and 8° respectively. However, the primary endings firing rate was silenced during faster shortenings (Edin and Vallbo 1990a) or larger amplitude shortening (Kakuda and Nagaoka 1998).

2.1.2 Golgi tendon organs

Golgi tendon organs (GTO) are the primary muscle force sensor in the body. They are located in the musculotendinous junction or in the tendinous inscription in series with the force producing muscle fibres (Jami 1992). GTOs are encapsulated afferent endings of 0.2-1mm in length and approximately 0.1mm in diameter (Jami 1992). In the Golgi tendon organ, the sensory ending of a group Ib nerve fibre is entwined amongst tendonous strands from 10-20 motor units and any given motor unit connects to 1-6 tendon organs (Prochazka 1996).

Feedback control studies on tendon organs have shown that they exhibit a high-pass filtering property (Houk and Henneman 1967), thus the dynamic component of the force signal is passed on more easily than the static. In other words group Ib afferents modulate their fire rates more when the load changes than for the static load.

The Golgi tendon organs have an autogenic disynaptic inhibitory pathway to the motoneuron and since they have a high threshold of activity to imposed muscle stretches in relaxed muscles, it was first thought that GTOs worked as overload

sensors. However, recording the Golgi tendon organ activity during muscle contractions revealed that the working range for the ensemble of the GTOs span the full physiological range of muscle forces (Houk and Henneman 1967; Crago *et al.* 1982; review see: Jami 1992; Prochazka 1996). Furthermore, the role of the Golgi tendon organs during walking has changed as both disynaptic and oligosynaptic excitatory pathways to the motoneurons have been reported during locomotor tasks (Conway *et al.* 1987; Pearson and Collins 1993; Gossard *et al.* 1994; Whelan *et al.* 1995b; Donelan *et al.* 2009; more information below 2.2.1).

The GTOs also exhibit several nonlinearities in their signalling relationship to force. First, at low active forces, ‘the beating effect’ or steps in the signalling of load from single GTOs can be observed as a result of muscle fibres are recruited. Second, active muscle fibres not inserting into the receptor may unload a given ending. Third, according to Prochazka (1996) it is unlikely that the force produced by muscle units attached to a given GTO reflect the total muscle force in a linear fashion. However, as Crago *et al.* (1982) showed, the combined discharge rate of several GTOs at moderate muscle forces is linear with the muscle force. The Golgi tendon organ is so far the sole sensor found to directly measure the force output of a given muscle. Although, it should be pointed out that other sensors do react to differences in load by indirectly being stimulated (Duysens *et al.* 2000); for example the muscle spindle afferents react to increased muscle stretch by an increase in force, the cutaneous afferents of the sole of the feet react to the skin stretch/compression related changes in loading, also skin afferents surrounding joints react to joint rotation imposed by the increased force.

2.1.3 Skin afferents

In the human skin there exists a variety of different mechanoreceptors. These receptors have both tonic and phasic responses to stimuli and respond to joint movement. Most of the mechanoreceptors, except for the free nerve endings, have a sensory body whose morphology sets the receptors response and the receptive field. Meissner’s corpuscles, Merkel disks and free nerve endings are present in the human glabrous skin and in the subcutaneous tissue also Pacinian corpuscles and Ruffini endings. In the hairy skin the mechanoreceptors are follicle endings, Ruffini endings, Pacinian corpuscles and free nerve endings. Ruffini endings are slowly adaptive and have large receptive fields giving them the potential for signalling joint rotation as they are sensitive to skin stretch across the joint (Burke *et al.* 1988). Stretching the skin overlaying finger joints has been shown to create an illusion of movement (Edin and Johansson 1995; Collins and Prochazka 1996), which reinforce the proprioceptive role of skin afferents. This proprioceptive role of skin afferents also translates to the human ankle joint, where the population vector (Georgopoulos *et al.* 1986) of in vivo recorded cutaneous afferents was correlated with ankle position (Aimonetti *et al.* 2000). Also the discharge rates of slowly adapting skin afferents in the human hairy skin surrounding the knee was found to provide high-fidelity information about knee joint movement. Though there is increasing evidence involving skin afferents in human proprioception, the

involvement of cutaneous feedback in the motor control of the lower leg muscles in walking human is still uncertain.

2.2 Role of afferent feedback in locomotion

This short review will be directed mainly on the spinal segmental feedback systems as this is the topic for this dissertation. The fundamental knowledge of afferent integration in animal models gives a sound basis for research questions posed for human bipedal walking. Even though, human locomotion is different from quadruped, hexapod and other forms of locomotion some of the basic principles may be common. Furthermore, when studying human bipedal gait we are restricted to minimally invasive procedures, and therefore we cannot replicate some of the preparations commonly studied in animal models. Hence, I will start by introducing some of the knowledge of the afferent feedback mechanisms for the cat.

2.2.1 Evidence from cats

Within the spinal cord the afferents connect with the efferent motoneuron through mono- and oligosynaptic connections and also as described above through the rhythm generating network. The complexity of the spinal network is such that we are still far from a complete picture of the interconnections, but extensive work has mapped the major afferent projections involved in afferent feedback (for review see: Jankowska 1992; Rossignol *et al.* 2006). Figure 2.1 presents a schematic view of main features of the spindle and GTO pathways.

Sensory feedback in the cat is not essential for generating locomotion. Even after complete deafferentation of one limb the animal quickly recovers the ability to locomote (Wetzel *et al.* 1976). However, the sensory information is essential for adapting the locomotor output to more demanding walking conditions in both cat (Bouyer and Rossignol 2003) and man (Blanchette and Bouyer 2009) and to entrain the spinal rhythm-generating network to switch phases (Grillner and Rossignol 1978). It is impressive how the spinal animal can adapt the phase transition to the speed of a treadmill and also switch between different modes of locomotion without the supra-spinal influence (Forssberg *et al.* 1980). Length sensitive afferents from the hip flexors (Grillner and Rossignol 1978; Hiebert *et al.* 1996) and also group I afferent from leg extensors (Duysens and Pearson 1980; Conway *et al.* 1987; Whelan *et al.* 1995a; Whelan *et al.* 1995b) have been implicated in assisting in phase transitions.

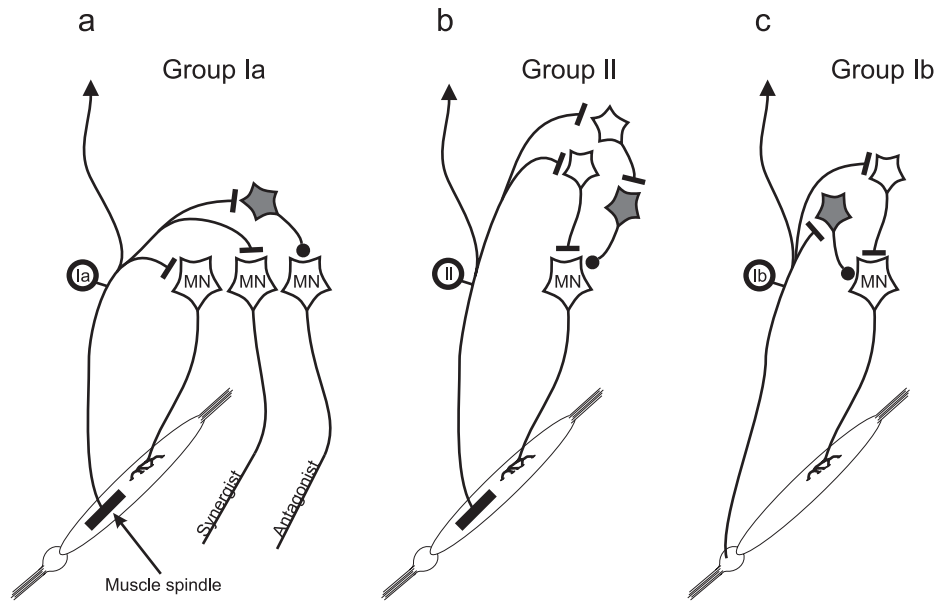


Figure 2.1: Schematic view of the main features of group Ia, II and Ib projections on the homonymous motoneuron.

a The group Ia pathway originates in the primary endings of the muscle spindle which are mostly velocity sensitive. The Ia fibre has an excitatory monosynaptic projection to the homonymous motoneuron and synergists motoneurons (MN). It also projects through inhibitory motoneurons to the antagonist motoneuron. All these projections are also under both excitatory and inhibitory actions from supraspinal centres. **b** The group II pathway also originate from the muscle spindles but the secondary endings are mostly length sensitive. The group II fibres projects on the homonymous motoneuron through excitatory disynaptic pathway and a trisynaptic inhibitory pathway. **c** The group Ib pathway originates from the Golgi tendon organ in the junction between the muscle fibres and the aponeuroses. During walking the group Ib pathway has a predominant excitatory disynaptic connection to the homonymous motoneuron, but also more indirect excitatory connections exist through the CPG (Gossard *et al.* 1994). During non-walking tasks the action of the group Ib pathway is inhibitory through a disynaptic pathway on the homonymous motoneuron.

Sensory feedback is also contributing to the generation of the locomotor activity. The sensory contribution to the locomotor activity has been studied in different preparations including intact cats (Gorassini *et al.* 1994; Donelan *et al.* 2009), decerebrate cats (Guertin *et al.* 1995; McCrea *et al.* 1995; Hiebert and Pearson 1999; Stein *et al.* 2000; Donelan and Pearson 2004a) and spinal cats (Hiebert *et al.* 1994). In all theses preparations sensory afferent contribution from the large myelinated proprioceptive afferents is responsible for the generation of a large part of the locomotor activity. As an example, when a spinal or intact animal steps into a hole, a large reduction in the locomotor activity is found (Hiebert *et al.* 1994; Gorassini *et al.* 1994). This reduction in locomotor activity has been hypothesised to be related to the loss of excitatory proprioceptive drive. This hypothesis was strengthened as the reduction in locomotor activity was not present when group I afferents were electrically excited (Hiebert *et al.* 1995) or when the ankle extensors were artificially loaded (Hiebert and Pearson 1999).

Also, a substantial part of the ankle extensor activity was attributed to sensory feedback as the isolated ankle extensors were stretched similarly as during walking in walking decerebrate cat. It is clear from the aforementioned investigations that the central locomotor drive is significantly enhanced by positive feedback from proprioceptive afferents to increase the extensor activity during the stance phase of the gait cycle, for review see (Pearson 2004).

As the locomotor activity of the extensor muscles during walking is to a large degree mediated through sensory feedback it is important to determine which afferents contribute to the feedback loop. During walking there is a reversal from inhibitory to excitatory feedback from the force sensitive Golgi tendon organ afferents (group Ib pathway) during the extensor phase, (Conway *et al.* 1987; Pearson and Collins 1993; Whelan *et al.* 1995b). During locomotion in spinal cats the electrical activation of group I afferents resulted in a significant increase of locomotor activity, while selective excitation of group Ia did not increase the locomotor drive (Pearson and Collins 1993). Also, proprioceptive feedback has been investigated in intact conscious cats and in cats where the gastrocnemius medialis was isolated by denervation of other ankle extensors (Donelan and Pearson 2004b; Donelan *et al.* 2009). By rapidly decreasing the ground reaction force one of the hind limbs and measuring the gastrocnemius activity it was confirmed that nearly 30% of the locomotor activity in over-ground walking was contributed by positive group Ib mediate force feedback (Donelan *et al.* 2009). Furthermore, during sloped walking the muscle activity was not strongly correlated to model estimates of either group Ia (stretch) or group II (length) afferents.

As highlighted above, there is now very convincing evidence for a proprioceptive contribution to the extensor locomotor activity in cats. However, the extrapolation of these results to human bipedal walking must be made very carefully as there are substantial differences between quadruped and human bipedal walking (Nielsen 2003). Bipedal walking is in essence a continuous controlled fall as the centre of mass is outside the base of support during walking. This inherent unstable system puts a higher demand on the control of locomotion. While forward propulsion in quadruped walking is shared among the hip, knee and ankle joints, in bipedal walking the ankle joint has a more pivotal role. The human ankle is dorsiflexed throughout the stance phase while ankle extensors are eccentrically contracted. In cats the range of motion of the ankle extensors is smaller which may make force feedback more suitable while in human potentially length feedback also plays a major part (Nielsen 2003).

2.2.2 Evidence from human experiments

Many studies have investigated the contribution of afferent feedback to the activation of leg muscles during human walking. The contribution was assessed by increasing the sensory feedback by mechanically or electrically perturbing the subjects and the resulting change in muscle activity was taken as evidence for the

sensory contribution. A multitude of studies have for example used the electrically induced Hoffmann-reflexes to investigate the spinal cord excitability and integration of afferent information (e.g. Morin *et al.* 1982; Capaday and Stein 1986; Crenna and Frigo 1987). The H-reflex was shown to be highly modulated during the step cycle, with the highest values during the stance phase (Capaday and Stein 1986). This was later taken as evidence for the same pathways being active in the contribution to muscle activity in the normal unperturbed situation.

Also mechanical perturbations have been used to assess the afferent contribution during walking. Dietz *et al.* (1984) used acceleration of the belt on a treadmill to mechanically stretch lower leg muscles during walking. This whole body perturbation was enough to elicit reflex activity in the plantar flexor muscles and the increased activity was taken as evidence for afferent contribution to the locomotor activity. More focused stretches of the ankle joint were performed some years later using a pneumatic piston that dorsiflexed the foot in stance (Yang *et al.* 1991). From these experiments it was proposed that nearly 30–60% of the soleus locomotor activity is contributed to by feedback from velocity sensitive group Ia afferents. It was also noted that this response was, as the H-reflex, modulated during the stance phase. However, the perturbation velocities of these experiments were dispersed (40–100°/s) and only in some cases could the peaks of the short- and the medium-latency response (SLR and MLR respectively) be seen.

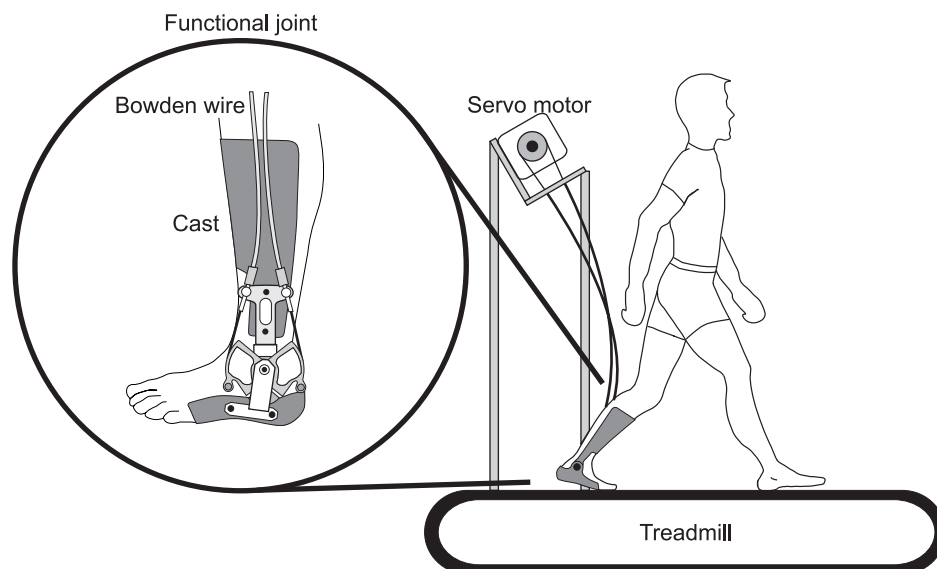


Figure 2.2: The portable stretcher, adapted from (Sinkjaer *et al.* 1996).

Andersen and Sinkjaer (1995) developed an apparatus for inducing rotational perturbations around the ankle joint called the portable stretcher, see Fig. 2.2. This machine used a powerful electrical servo motor connected through flexible Bowden cables to a functional joint secured around the ankle (Andersen and Sinkjaer 2003). The use of this machine enabled perturbations to be presented throughout the step cycle and for the stretches of the plantar flexor muscles to be

better controlled in velocity and amplitude. The device was first used to characterise the stretch reflex activity in the soleus muscle throughout the stance phase ($\sim 250^\circ/\text{s}$, 8° dorsiflexion), where it was found that the highest amplitudes of the stretch reflex were in the stance phase (Sinkjaer *et al.* 1996). The authors suggested that stretch reflex activity could contribute substantially to muscle contractions during gait.

These studies give valuable insight into how corrective responses to fast unexpected perturbations are processed in the spinal cord. However, the extension of these results to the sensory processing of normal unperturbed walking should be done carefully. Morita *et al.* (1998) showed that the temporal characteristic of the afferent volleys is key in the neural integration. The effectiveness of the presynaptic inhibition on the monosynaptic group Ia pathway is significantly altered dependent on the temporal dispersion of the afferent activity. Thereby, the rapid stretch of a muscle or the electrical analogue, the H-reflex, can only provide evidence for spinal integration of temporally similar responses. Hence, the stretch reflex data may be valid for corrective responses to unexpected perturbations but may not give an estimate of the afferent contribution to the ongoing EMG activation in unperturbed steps (Sinkjaer *et al.* 2000; Nielsen 2002; Nielsen and Sinkjaer 2002).

Sustained enhancements or reductions of the sensory feedback have also been used to investigate the contribution of certain pathways to the locomotor activity in human walking. To investigate load feedback during gait, body weight support was used to change the forces acting on the supporting limbs (Harkema *et al.* 1997; Stephens and Yang 1999). Stephens and Yang (1999) investigated sustained and transient loading of the subject by 30% of body weight during treadmill walking. They found that the increase in body load produced increases in the muscle activity of the soleus muscle. Similarly, Harkema *et al.* (1997) evaluated the locomotor activity in leg muscle during sustained changes of body weight support in spinal cord injured patients. A correlation between the muscle activity and the amount of load on the supporting limb was found and the authors concluded that that positive load feedback was contributing to the locomotor activity. The group Ib pathway from gastrocnemius medialis to the soleus motoneurons was also recently investigated by Faist *et al.* (2006). By electrically conditioning the group Ib afferents from the GM and monitoring a soleus H-reflex they showed that the Ib inhibition is only present when the leg is not loaded and that a facilitatory group Ib pathway may become opened during gait. The role of cutaneous sensory information has also been investigated during walking by reducing the sensory information from different parts of the sole of the foot using an ice intervention (Nurse and Nigg 2001). The pressure distribution on the sole of the foot during stance changed after ice intervention moving the centre of pressure away from the anaesthetised area. This difference in centre of pressure was also followed by a subsequently change in soleus muscle activity which the authors claimed to

indicate that feedback from cutaneous afferents is important in regulating and modifying the soleus locomotor activity.

These studies give an indication of the involvement of proprioceptive feedback in the control of locomotor output during walking. However, it is arguable whether or not these changes in muscle activity stem from the changes in proprioceptive feedback or is a result of changes in descending drive. Except for the transient changes in body weight support (Stephens and Yang 1999) and the electrical stimulations applied by Faist *et al.* (2006), the studies enhance or reduce the sensory activity over a prolonged period of time. The changes in locomotor output may therefore be a compensatory adaptation in the descending drive to the change in sensory feedback. Instead of using sustained enhancements or reductions of the sensory information, transiently removing the sensory feedback does limit the possibility for the descending drive from interfering with the measurement. It was hypothesised that a rapid plantar flexion perturbations during the stance phase would effectively unload the soleus muscle-tendon unit, stopping the natural eccentric contraction, and thereby transiently cesses or significantly decrease the spindle and Golgi tendon organ afferent activity (Sinkjaer *et al.* 2000). By using the portable stretcher, Fig. 2.2, Sinkjaer *et al.* (2000) induced such rapid plantar flexion perturbations in mid stance while walking on a treadmill. The response in the soleus locomotor activity was a sharp depression of approximately 50% as compared to non-perturbed activity. This depression was not the result of reciprocal inhibition from the stretch of the TA tested by peroneal nerve block. Furthermore, they used ischemia to block the largest group I afferents to show that the cessation of the velocity sensitive group Ia afferents were not significantly contributing the large depression in locomotor activity. These results were extended by Grey *et al.* (2004; 2007) by excluding feedback from cutaneous afferents as a major source of the response and emphasising the potential role of positive force feedback from group Ib afferents in the generation of the locomotor activity. These experiments clearly show that sensory afferent activity from proprioceptors contributes to the generation of locomotor activity in human walking. However, this does not automatically infer that the same afferents are also actively modulating the muscle activity during walking as the afferent input could gait some central drive to the motoneuron.

As highlighted above, when investigating afferent feedback's effect on the locomotor output it is crucial not to change the feedback signals in such a way that they would be integrated differently than what is normally the case in over-ground walking. Under this assumption, Mazzaro *et al.* (2005; 2006) attempted to investigate the afferent mediated modulation of the locomotor during treadmill walking. By imposing slow velocity, small amplitude enhancements and reductions of the ankle dorsiflexion velocity during stance phase they found, respectively, increments and decrements of the soleus muscle activity. Local anaesthetic depression of cutaneous afferents from the foot and ankle showed that the modulation of the locomotor activity was not dependent on cutaneous

afferent input. On the other hand, an ischemic depression of largest group I afferents and tendon vibration indicated that group Ia afferent did significantly contribute to the increase in locomotor activity when the dorsiflexion velocity was enhanced, but not when it was reduced. The reduction of locomotor activity was suggested to be mediated by reduction in feedback from group II and possibly group Ib afferents. However, these studies and most studies trying to discriminate the afferent contribution in human walking have used the gross measure of muscle-tendon length and lengthening velocity to estimate changes in spindle afferent feedback. The optimal would be a direct measurement of the afferent signals to determine the gain of the feedback loop; unfortunately this is impossible in human with current recording techniques. A recent study using ultrasonography of the soleus muscle fascicles showed in standing that the elastic tendon in series with the muscle could make length changes at the muscle fibre level impossible to detect by only studying the muscle-tendon length (Loram *et al.* 2004). Also in walking, the muscle fascicles of the bi-articulate gastrocnemius medialis muscle were not eccentrically contracted in mid stance as the muscle-tendon length indicated (Ishikawa *et al.* 2005). This highlights some of the challenges that are met when indirectly studying the afferent contribution and modulation of locomotor activity in humans.

Furthermore, most studies on afferent feedback have been conducted with treadmill walking subjects, as it requires less laboratory space, steady-state walking speeds are selectable and consecutive trials are easily recorded. However, the validity of comparing treadmill walking to over-ground walking has been debated for some time. Treadmill locomotion has been associated with changes in ground reaction forces (White *et al.* 1998), kinematics (Wank *et al.* 1998), and shorter stride length and increased cadence (Pearce *et al.* 1983). On the other hand, recent studies contradict some of these claims (Lee and Hidler 2008; Riley *et al.* 2007) and theoretically there is no mechanical difference between the two tasks (van Ingen Schenau 1980). However, changes in the instantaneous belt speed may influence the kinematics (Savelberg *et al.* 1998) and treadmill walking has been suggested to artificially reduce some of the natural variability in the locomotor patterns (Wank *et al.* 1998; Dingwell *et al.* 1999). Adding to this confusion, was a recent study showing that quiet standing posture is affected by treadmill walking but not over-ground walking (Zanetti and Schieppati 2007). In conclusion, the debate as to the difference between the two conditions is not yet resolved which emphasises the need to validate the role afferent feedback also in the over-ground walking condition.

2.3 Project Goals

The work of Mazzaro *et al.* (2005; 2006) show that the locomotor activity is modulated with respect to the imposed dorsiflexion perturbations. Even though, these imposed changes in ankle trajectory are within what can be found during unperturbed steps, the nature of imposing a certain ankle trajectory may well influence the afferent firing rates differently than what is normally occurring when stepping on irregularities in the ground. As the afferent firing characteristics is currently impossible to measure during walking, we cannot exclude the possibility that more burst like activity is induced by the imposed ankle dorsiflexion velocity enhancements that is not directly visible in the motor output. To remove this potential source of error, it is important to make the investigations of the afferent contribution in as natural and unrestricted environments as possible. This is further emphasised by the potential biomechanical and kinetic differences between treadmill and over-ground walking. The information gained from the investigations using “unnatural” perturbations run the risk of only being valid for just those types of perturbations or be confounded by startle responses or corrective responses. Therefore the obvious continuation is to bring the experiments to an over-ground setting using perturbations commonly encountered during walking. In essence we have to ask: In *over-ground* human walking does afferent feedback modulate EMG activity in plantar flexors?

The study by Sinkjaer *et al.* (2000) clearly shows that proprioceptive afferents contribute to the locomotor activity in human walking. However, it is much debated which afferent pathways contribute to the ongoing EMG activity during human walking. A comparison between the latency of the unloading response and stretch reflex components indirectly show involvement of either group Ib or group II afferents in mid to late stance (Grey *et al.* 2004). Other studies have suggested the involvement of both group Ia and group II, (Mazzaro *et al.* 2006; Mazzaro *et al.* 2005), and of cutaneous (Nurse and Nigg 2001). However, the effects of load sensitive group Ib afferent contribution on the modulation of the muscle activity has not been sufficiently investigated due to difficulties separating force from length changes.

The aims of this Ph.D. project were:

- I. To determine which proprioceptive afferents contribute to the locomotor activity in the plantar flexor soleus muscle.
- II. To determine if afferent feedback modulates the plantar flexor locomotor activity in *over-ground* human walking.
- I.I To investigate which afferents play a part in this modulation of plantar flexor locomotor activity in over-ground human walking.

These objectives were investigated in four research studies:

Study I: “*Within-step modulation of leg muscle activity by afferent feedback in human walking.*” J. Physiol. 2008, 586(Pt 19):4643–8.

The aim of this study was to determine if sensory feedback modulates the triceps surae locomotor activity during the stance phase in unrestrained over-ground walking. Minimal variations in the inclination of a stable support surface for one step was used to alter the sensory feedback during the stance phase as it would be when walking on a slightly uneven surface. The hypothesis was that the alterations in sensory information from the foot and leg altered by stepping on the slightly inclined or declined surface would modulate the locomotor activity of the plantar flexor muscles within the step during over-ground walking. Stepping on the inclined surface increased the ankle dorsiflexion as well as the estimated Achilles tendon force and respectively decreased the ankle dorsiflexion and Achilles tendon force for the declined surfaces. The triceps surae was similarly correlated with the inclination of the supporting surface, while the dorsiflexion velocity and triceps surae muscle-tendon lengths were negatively correlated with the inclination of the support surface. These modulations of the triceps surae locomotor activity was not found in one tested deafferented subject, indicating that the modulations were neither vestibular nor visually in nature. These results suggest that the modulation of triceps surae muscle activity in the stance phase is most probably the result of feedback from length sensitive and/or force sensitive afferents.

Study II: “*Sudden drop in ground support produces force related unload response in human over-ground walking.*” J. Neurophysiol. 2009, accepted Jan 21.

The aim of this study was to investigate the contribution of afferent feedback to the soleus muscle activity in over-ground human walking. By rapidly reducing the ground reaction force during single support stance phase the natural dorsiflexion of the ankle and the force on the plantar-flexor muscles are drastically reduced. The hypothesis was that the transient reduction in proprioceptive feedback following the reduction in ground reaction force would, as for imposed plantar flexion perturbations during stance, result in a short latency depression of the soleus locomotor activity. The length sensitive afferent feedback was investigated by reducing the ankle excursion using an ankle foot orthotic. Both mid- and late-stance perturbations elicited a depression in soleus locomotor activity. The reduction of the ankle excursion reduced the overall soleus locomotor activity, but the late-stance depression following the reduction in ground reaction force remained. These results suggest that force related afferent feedback may be contributing to the locomotor activity in the soleus.

Study III: “*Load rather than length sensitive feedback contribute to soleus muscle activity during human treadmill walking*” submitted to J. Neurophysiol. 2009

The aim of this study was to investigate the effect of transient body weight support changes on the unload response and the stretch reflex response. The unload response is elicited by a rapid plantar flexion perturbation unloading the muscle-tendon unit of the soleus muscle resulting in a depression in the locomotor activity at latencies longer than the SLR and shorter than the MLR. The unload response has been proposed to depend on group II and/or group Ib afferent feedback. The stretch reflex medium latency response is mostly mediated by group II afferent feedback, but as the conduction velocity of group Ib afferents is higher than for group II, there exists a possibility that group Ib afferents also could contribute to the MLR. It was hypothesised that transiently reducing the load on the limb, by increasing body weight support, would reduce the group Ib afferent contribution which would be measured in a reduction of the unload response. Also, if group II afferents contribute to the unload response then a Tizanidine induced depression of the group II afferent pathway should significantly reduce the unload response. Furthermore, it was hypothesised that if group Ib contribute to the MLR response then it would also be reduced by the reduced load on the limb. Both the MLR and the unload response was significantly decreased when the body weight support decreased the load on the limb. At the same time the SLR response did not change indicating that the excitability of the motoneuronal pool was not significantly decreased by the change in body weight support. Also, the depression of the group II afferent pathway measured by a significant decrease in MLR did not affect the late stance unload response. These results indicate that the medium latency response of the stretch reflex is to a small extent mediated by feedback from group Ib. Furthermore, the unload response does show a dependency on the group Ib afferent contribution indicating that force/load feedback does contribute to the locomotor activity in the stance phase of human walking.

Study IV: “*Afferent contribution to locomotor muscle activity during unconstrained over-ground human walking: an analysis of triceps surae muscle fascicles*” submitted to J. Neurophysiol. 2009

The aim of this study was to investigate the within step modulation of plantar flexor locomotor activity and the contribution of afferent feedback to the locomotor activity in unrestrained over-ground walking. More precisely, the muscle fascicle lengths of the soleus and gastrocnemius medialis muscle were acquired using high-speed ultrasonography to better estimate the effect of the perturbations studied in study I and II on the spindle afferent output. It was hypothesised that the within-step modulation of locomotor activity would coincide with changes in muscle fascicle and tendon length. Furthermore, it was hypothesised that if group II length sensitive afferents do contribute to the

locomotor activity, the change in the fascicle length should occur so that sufficient time is left for the synaptic transition and integration before the onset of the response in the locomotor activity. Indeed, the muscle fascicle lengths and Achilles tendon length were lengthened by stepping on the positively inclined surface and shortened by the declined surface. However, no change in the muscle fascicle lengthening velocity was noted which indicates that it is unlikely that feedback from group Ia afferents significantly modulate the locomotor activity during walking. The rapid decrease in ground reaction force shortens the soleus muscle fascicles. However, the onset of this shortening is significantly delayed with respect to the change in ground reaction force, indicating that it is more likely that the response is mediated by group Ib than group II afferents.

3 Study I

Within-step modulation of leg muscle activity by afferent feedback in human walking. af Klint R, Nielsen JB, Cole J, Sinkjaer T, Grey MJ. J Physiol. 2008 Oct 1; 586(Pt 19):4643–8.

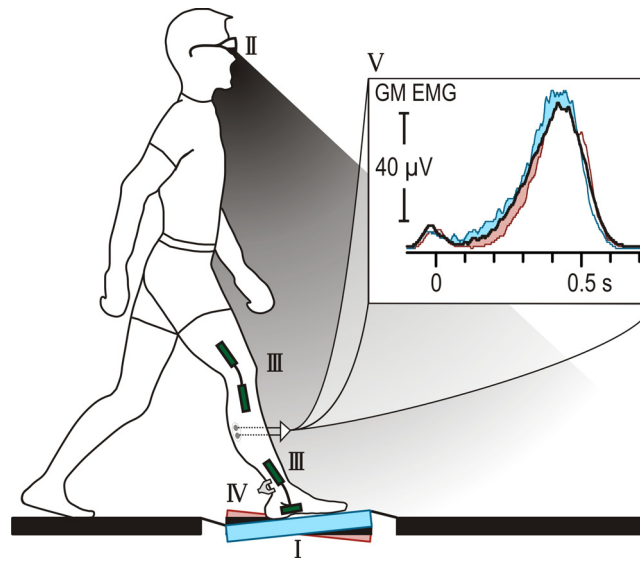


Figure 3.1: Experimental setup study I.

Subjects stepped on an inclined surface (I) in the middle of a 10 m walk-way. Knowledge of the platform's inclination was prevented by occluding the subject's view (gray area) with taped glasses (II). Ankle and knee rotation were recorded using goniometers (III) and Achilles' tendon force was estimated by an external buckle transducer (IV). Ensemble averaged ($n > 20$) electromyographic recordings from gastrocnemius medialis (GM) of a representative subject is presented for 0° (black), $+3^\circ$ and -3° (increase and decrease, respectively, in the EMG) degree inclination (V), showing modulation in muscle activity induced by the inclination of the platform. Adapted from (af Klint *et al.* 2008).

4 Study II

Sudden drop in ground support produces force related unload response in human over-ground walking. af Klint R, Nielsen JB, Sinkjaer T, Grey MJ. Neurophysiol. Jan 2009

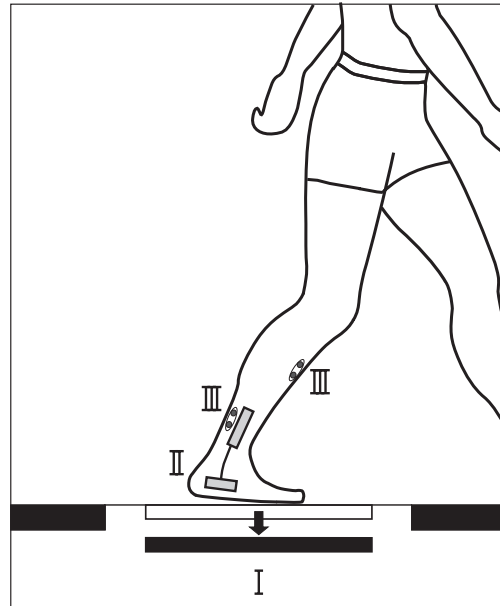


Figure 4.1: Experimental setup study II.

Subjects walk at a self selected speed on a 10m walkway, stepping on a hydraulically activated platform (I) with the right leg. Joint rotation of the ankle was recorded throughout the step using goniometry (II). Surface EMG was recorded from the soleus and tibialis anterior muscles (III). On random trials the platform was accelerated downward with 0.8g at a preset timing in stance. Adapted from (af Klint *et al.* 2009).

5 Study III

Load rather than length sensitive feedback contribute to soleus muscle activity during human treadmill walking. af Klint R, Mazzaro N, Nielsen JB, Sinkjaer T, Grey MJ; (submitted)

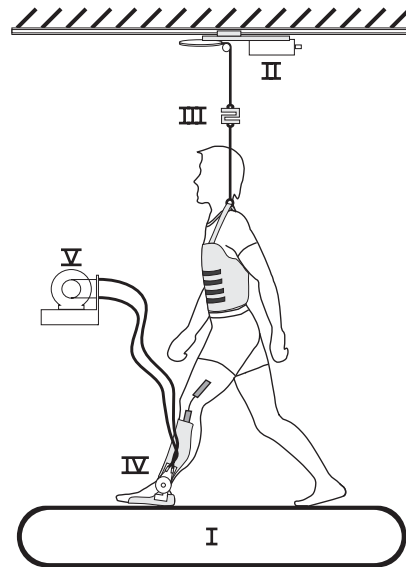


Figure 5.1: Experimental setup study III.

The subjects walked on a treadmill (I) at a self selected speed, while they were supported by a constant force body weight support (BWS) system (II). A strain gauge (III) in series with the BWS measured the lifting force as it was changed between 5% and 30% of body weight. The subjects were also fitted with a functional joint (IV) connected by Bowden wires to a computer controlled servo motor (V) that enabled fast rotation perturbations of the ankle.

6 Study IV

Afferent contribution to locomotor muscle activity during unconstrained over-ground human walking: an analysis of triceps surae muscle fascicles. af Klint R, Cronin NJ, Ishikawa M, Sinkjaer T, Grey MJ (Submitted)

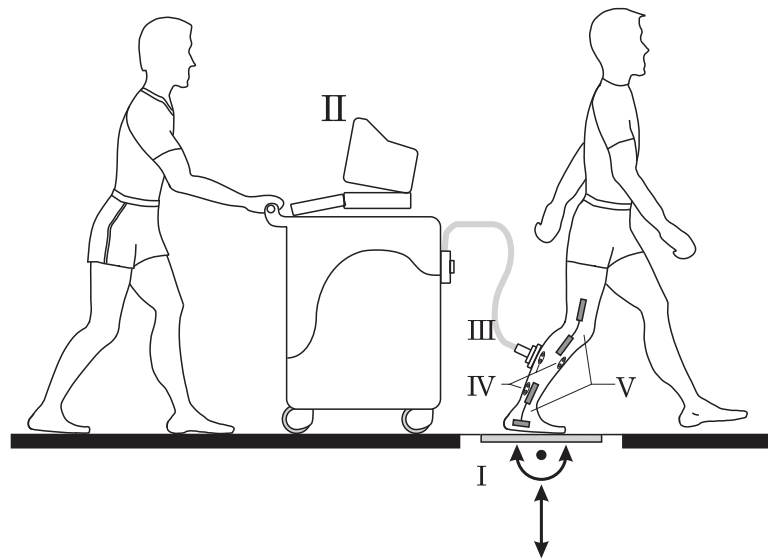


Figure 6.1: Experimental setup study IV.

Subjects walked over a hydraulically actuated platform (I) that on random could produce a rotation in the parasagittal plane or a controlled drop of 8cm at 0.9g acceleration. The subjects were instrumented with an ultrasound probe (III) positioned so that the soleus and gastrocnemius medialis muscle fascicles were acquired by the ultrasonograph (II). Synchronised with the ultrasound, surface EMG of soleus, gastrocnemius medialis and tibialis anterior (IV) as well as goniometry of the ankle and knee (V) was recorded.

7 Methodological considerations

Before a more careful discussion of the main result some of the limitations of the used methods should be highlighted. When studying human locomotion it is impossible with the current recording techniques to directly measure the afferent signals. Therefore, all the results and comparisons stated in this dissertation must be regarded as indirect. However, it also highlights the importance of animal models, and even though humans are the only mammal to use bipedal plantigrade walking as main form of locomotion it would be surprising if the fundamental feedback mechanisms are different.

It has been shown that reflex gains are task specific (e.g. Capaday and Stein 1986; Capaday and Stein 1987; Faist *et al.* 2006) and may change with the difficulty of the task (e.g. Llewellyn *et al.* 1990; Schneider *et al.* 2000). Therefore, all investigations were done during walking either over-ground or on a treadmill. Furthermore, as the temporal dispersion of the afferent signals affects the spinal integration of the signal (Morita *et al.* 1998), care was taken in the design of the experiment to make them mimic natural walking conditions or transiently remove or depress the afferent signals to avoid changes in the spinal integration.

A general assumption in the current dissertation is that the afferent activity of a specific pathway follows the changes in the sensory modality of that pathway, i.e. if the load on a muscle increases then it is assumed that the Golgi tendon organs and the innervating group Ib fibres increase their activity. The Golgi tendon organs do exhibit several nonlinear properties but are mostly sensitive to the forces exerted by the muscle (Prochazka and Wand 1980). Hence, the assumption is likely valid for the group Ib afferents. For spindle afferents the separation between length and velocity sensitivity is more complicated. Both muscle fibre lengthening velocity and muscle fibre length influences the activity of the group Ia and group II afferents (Matthews and Stein 1969b). However, the group Ia is significantly more sensitive to stretching velocity than the length of the muscle stretch, see background. Also, the sensitivity of the spindle afferents is regulated by gamma activation which can alter the response to the two modalities. This prevents a conclusive discrimination of the pathways involved solely dependent on the dynamics of the muscle and other methods should be combined to better discriminate between the two groups of spindle afferents. However, as a first estimate the muscle fibre dynamics may give a reasonable approximation of changes in the spindle afferent pathways.

7.1 *Mechanical perturbations*

The main form of investigation has been the use of mechanical perturbations giving rise to the recorded responses in the muscle activation. The current section will discuss the relevance of the employed perturbations.

7.1.1 *Mimicking natural variations*

The aim was to investigate the afferent modulation of locomotor activity as it is present in the everyday walking environment. In study I, and replicated in study IV, the paradigm mimics the natural variations in the walking surface by having the subject walk over a platform that was inclined prior to heel contact. We chose to use a minimal perturbation ($\leq \pm 3^\circ$) in order not to elicit any compensatory reflexes. Even though these small changes in inclination nicely replicate the conditions met when walking outside and hence also the modulation of locomotor activity to such conditions, they pose some challenges from an experimental design point of view.

The hydraulic platform was mounted flush in the floor of the laboratory and has a size of 60x60cm. This presents a difficult target to touch down upon when the view of the platform is blocked. Some subjects had more difficulty than others, but all succeeded after 5min of practice to touch down approximately centred on the platform. The approach to the platform was adjusted some 5m from the platform and the subjects were instructed to walk at a constant pace and thereby hitting approximately the same spot on the platform. Nevertheless, the fact that it took a conscious effort for the subjects to control their pace and that all the inclinations presented were by necessity centred on the platform may have made the subject more aware of the presented inclinations. However, when quantifying the subjects' awareness of the inclinations, the size of the inclinations were not large enough for the subjects to reliably determine the inclination, see Study I discussion.

As the platform rotation movement was initiated and finished prior to heel contact, it could not be adjusted to the foot position at heel contact. This will in itself induce some variance in the mechanical significance of stepping on the incline or decline. Stepping on the inclined platform induces an inversion or eversion of the ankle dependent on the internal/external rotation of the foot. Furthermore, the height at which the heel made contact with the platform may differ between the different platform rotations as it is proportional to the rotation and the distance from heel touch down to the centre of rotation.

van der Linden *et al.* (2007) specifically investigated the response to stepping on a surface with unexpected height. When the anticipated height of the foot fall was not met, the gastrocnemius medialis muscle activity was consistently increased at approximately 90ms after the anticipated foot fall. To induce this effect van der Linden *et al.* (2007) used a difference between anticipated and actual height of -5cm, i.e. the subjects stepped into a hole that was 5cm deep. Unfortunately, limitations in our recording techniques prevented a direct measurement of foot placement, and hence the influence of the difference in height. However, in our study this difference in height could maximally vary between ± 1.5 cm dependent on where on the platform the foot touches down. Anecdotally, from visual inspection the subject stepped consistently less than 10cm off the centre of

rotation relating to a difference in height of around $\pm 0.5\text{cm}$. Hence, the difference in height is a significantly smaller than what was used to elicit the response. Therefore, the difference between the anticipated afferent information and the actual information smaller for the current study and unlikely to contribute to the differences in activity reported for the inclinations.

Lastly, to avoid anticipatory effects the subjects wore taped glasses blocking the platform from the field of view. By obscuring the field of view of the subject, we did run the risk of them walking more cautiously at the start of the experiment. The 5 min of accommodation is likely to have attenuated this effect as the subjects became more comfortable with the situation. However, foot clearance may have increased by wearing the taped glasses. As this potential change in kinematics would be comparable for all the presented platform inclinations, it is unlikely that this would have affected the reported modulation of the locomotor activity.

7.1.2 Remove afferent feedback

Unloading the Triceps surae muscle-tendon complex during walking should decrease the muscle spindle and Golgi tendon organ firing rates as the muscle spindles are less stretched and the force on the muscle-tendon complex is reduced. Hence, the response to this removal/reduction of afferent drive can be studied. These experiments were inspired by the foot-in-hole experiments used in cat preparations (Gorassini *et al.* 1994). However with a major difference being that the perturbations are not performed at heel contact but later in stance when the muscle-tendon complex is loaded. The unloading of the Triceps surae muscle-tendon complex was performed using two different approaches, both having its advantages and disadvantages. The original method, first mentioned in Sinkjaer *et al.* (2000), utilises a functional joint capable of rotating the ankle at any stage in the walking cycle. This apparatus, the semi-portable stretcher, directly plantar flex the ankle joint and thereby producing a shortening of the Triceps surae muscle-tendon length, used in Study III. This apparatus is best suited for treadmill walking and was not used for the over-ground investigations of the unload response, study II and study IV. In these studies the Triceps surae muscle-tendon complex was “passively” unloaded, i.e. there was a decrease of the forces counteracting the muscle contractions but no imposed shortening of the muscle-tendon complex.

The semi-portable stretcher can induce consistent, in velocity and amplitude, dorsiflexion and plantar flexion perturbations while the subject is walking on a treadmill. The functional joint does increase the mass of the limb, however previous investigations have reported no differences in kinematics (Andersen and Sinkjaer 1995). Though the fast plantar flexion and dorsiflexion rotations are applied at the ankle joint the added torque will in some cases generate movements at the knee. This is especially true for plantar flexion perturbations in mid to late stance, as the plantar flexing torque will generate an extension of the knee. Potentially, this could be one of the reasons why unload responses are not

consistently seen in the gastrocnemius muscle as an extension of the knee could trigger an increased stretch related activity in the biarticular gastrocnemius.

Otherwise, the portable stretcher provides a stronger and more focused perturbation than the movable platform. The platform perturbations rely on the removal of ground reaction force to initiate the rotation of the ankle, and therefore it is also a full body perturbation and not focused on the ankle joint. On the other hand, all perturbations by the portable stretcher are rotations of the ankle joint, hence directly change the muscle-tendon length but at the same time changing the torque around the ankle. Therefore, the rotational perturbations per se dissociate between changes in muscle-tendon length and force on the tendon. This is different for the platform drops, as the perturbation rapidly decreases the ground reaction force, i.e. the force on the ankle extensors are directly influenced, while the shortening of the muscle fibres may occur later. Furthermore, the muscle-tendon shortening can be reduced by limiting the rotation of the ankle joint by an ankle foot orthotic, see study II. Therefore, the rapid reduction of the ground reaction force, by dropping the platform, may help to dissociate the length and force feedback to the spinal cord.

Perturbations of gait using the movable platform are restricted in the location, as the platform is built into the flooring of the lab. This can potentially make the subjects adapt to the platform movements. This risk of adaptation was reduced by adding several control steps, non perturbed, between all perturbed steps. However, in some subjects no clear reduction in soleus activity was seen when the perturbations were presented in mid stance (7 out of 38 tested subjects). The lack of a mid stance response is unlikely to be contributed to by the subjects adapting as the late-stance perturbations still induced a short lasting depression in the same subjects.

7.1.3 Transient body weight support

In study III, the effect of transiently changing the body weight support was investigated on the unload response and the stretch reflex. Previously, sustained body weight changes have been used to show the importance of load feedback. However the subjects may adapt to sustained BWS. This was avoided in the current setup by changing the body weight support between two levels every 3-5 steps thereby limiting any adaptation occurring.

The two levels of BWS chosen were 5% and 30% of body weight. As the changes in BWS are drastic, occurring over less than one step cycle, it is important that the cable supporting the subject is positioned vertically directly over the subject. If this is not the case it is likely that the increased lifting force would induce postural responses. To ensure that this does not occur the lower level of BWS was set to a sufficient amount of force to make the BWS device follow the subject's movements on the treadmill. We also attempted similar experiments during over-ground walking but quickly found that even though the BWS-system was

mounted on low friction rails, the acceleration at the start and end of the runway and the friction prevented the BWS-system to follow the subject at low levels of BWS. The higher level of BWS, 30%, was chosen as to compare with previous experiments using sustained BWS (e.g. Stephens and Yang 1999; Grey *et al.* 2002; Mazzaro *et al.* 2006).

Ideally the perturbations used to probe the afferent feedback should be performed during or directly after the change in BWS. In preliminary experiments it was attempted to induce the unload response during the first step after the change in BWS. However the variability of the ankle trajectory and the soleus EMG prevented a good measurement. As no significant change in the magnitude of the muscle activity was found between the first and second step after change of BWS, it was decided to induce the unload response on the second step. Even though an adaptation to the BWS may occur, the subjects are most likely not fully adapted to the BWS during the second step.

7.2 Deafferented subject

In study I, we were fortunate to have the possibility to recruit a subject who lacks large myelinated afferents below the neck. At the age of 19, this subject, IW, got a stomach flu that triggered an autoimmune response which attacked his large myelinated afferents. After a few days, he had lost all proprioception and light touch below the neck, sparing deep touch, nociception and thermal sense. After several years with physiotherapy he was able to relearn how to walk using visual feedback and a high degree of conscious control of his muscles.

IW is to the author's knowledge one out of two reported cases that after large myelinated neuropathy is still able to walk. To compensate for his lack of proprioception, IW uses different tricks. For example, his thermal sense in the armpit can indicate if the shoulder is raised or not (personal communication, May 2006), also muscle fatigue can indicate the weight of object held (Cole and Sedgwick 1992). However, during dynamic movements IW will have to rely on other sensory information.

The fact that IW has adapted his muscular control to the lack of some sensory information makes a direct comparison to able bodied volunteers complicated. On the other hand, the same biomechanical constraints apply to IW's walking as to the able bodied. The effect of removing proprioceptive feedback on the muscle activity during walking cannot be investigated in a deafferented subject as the control scheme has adapted to the lack of proprioception. However, the influence on the spared sensory pathways and their importance to the locomotor control would most probably increase. This was also seen for the vestibular reflexes in IW which were normally modulated but increased with respect to able bodied subjects (Day and Cole 2002).

7.3 *Achilles tendon force sensor*

In study I an external buckle transducer was used to estimate the Achilles tendon force (ATF). The sensor design was based on the in vivo buckle transducer (Salmons, 1969; Komi *et al.*, 1987) and an external tendon-clamp transducer (Berger *et al.*, 1982). The Achilles tendon force sensor works by clamping the tendon on the surface of the skin within an E-buckle transducer, see Fig. 7.1. The lateral forces on the clamp, measured by strain gauges mounted on the connecting beam, correlates with the tension in the Achilles tendon. Therefore, an estimate of the difference in tension in the Achilles tendon can be seen just from the difference of the output signal.

The foot decelerates drastically at heel contact, which puts significant constraints on the design of the ATF-sensor. The sensor design therefore emphasised a small mass and moving the centre of mass close to the tendon. This way, the shear forces were minimised which otherwise would make the device loose sensitivity and eventually fall off. Also, the fast deceleration at heel contact together with the relatively low tension on the tendon makes measurements at heel contact unreliable.

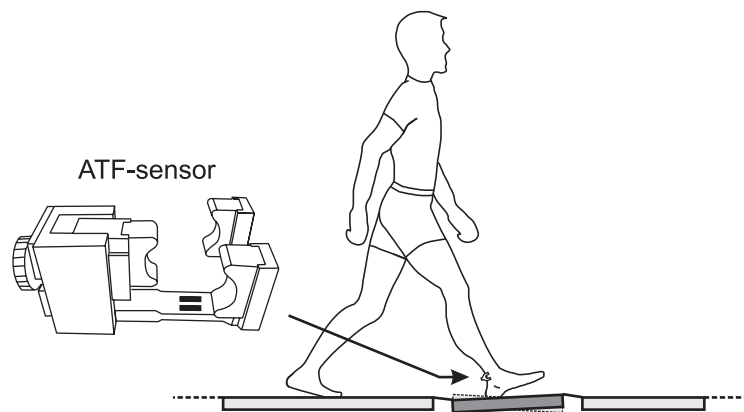


Figure 7.1: Achilles tendon force transducer and placement

The sensitivity of the sensor is increased with the amount of lateral compression on the tendon when fitting the device on the subject. Also, with a tighter fit around the Achilles tendon the sensor is less likely to slip off. However, there is a trade off in the mounting of the sensor as the tighter it is secured the more uncomfortable it will be for the subject. Whenever placing the ATF-sensor care was taken so that the placement and lateral forces on the tendon were just enough for a good recording, so as to prevent the sensor from changing the subject's kinematics while preserving sensitivity. More to, it was used for a limited period of time as the sensitivity does decrease over time both from slippage and likely due to changed elasticity of the surrounding tissue. Hence, perturbations were

always presented with a uniform distribution, making the loss of sensitivity affect the different perturbations equally.

Preliminary studies on two lower leg specimens were carried out to infer that the sensor may estimate the tension on the Achilles tendon, see Fig. 7.2a. The sensor output is clearly correlated to the tension on the tendon, see Fig. 7.2b. However, as the ankle rotates the tendon is compressing the tissue between the ankle joint and the tendon. This compression increases the sensitivity of the ATF-sensor, see Fig 7.2c-d. Dependent on the specimen used the estimate for the normalised force differed between 3-5%/°. In study I the ankle dorsiflexion changed when the subjects stepped on the platform. This change in dorsiflexion would change the sensitivity of the ATF-sensor in such a way that the measured differences between the inclinations of the platform could potentially be the result of the dorsiflexion and not of an increase in Achilles tendon load. To investigate this effect the relative difference of the ATF-sensor output to 0° inclination was calculated and compensated for the dorsiflexion induced change in sensitivity, lower bound 3%/° and upper bound 5%/°. The repeated measures analysis of variance showed for both the upper and lower estimate a clear significance for the perturbation (3%/°: $F_{17,3}=34.15$, GG $p<0.001$; 5%/°: $F_{17,3}=10.50$, GG $p=0.001$). The post-hoc Tuckey-Kramer test showed for the 3%/° compensation a clear difference between all positive and negative inclinations ($p=0.001$). Also for the 5%/° compensated output, the modulation of the sensor output with the positive and negative platform inclinations remained, but at lower significance. This initial verification of the ATF-sensor shows that the Achilles tendon tension was modulated with the inclination of the platform in study I. However, sensor would benefit greatly from a better calibration method, potentially using ankle goniometry to compensate for losses in sensitivity.

This sensor has definite constraints in its use, as highlighted above. Even so, it is still of value to be able to non-invasively characterise the changes in force level at the Achilles tendon. The estimate of the forces can be an indicator of how this information is processed. Kinetic estimates can also be used; however, they rely on motion capture and force-plate information, limiting the experimental setup and are sensitive to errors in the kinematic model and accuracy of the motion capture.

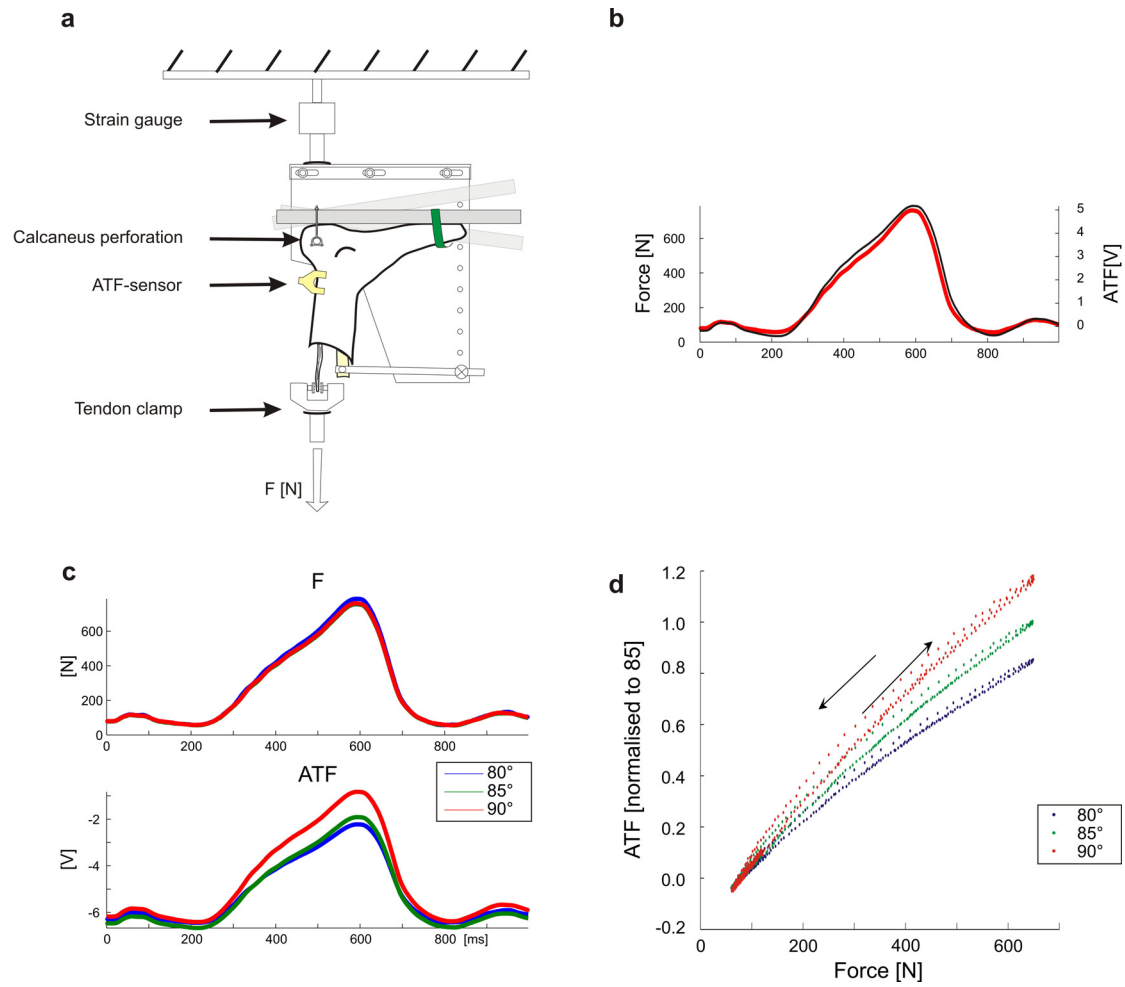


Figure 7.2: Achilles tendon force transducer Preliminary test-data

a Test of the ATF-sensor on lower leg specimen. The tendon was secured to a hydraulic piston and the foot was secured to a strain-gauge (material testing machine: Dual-Mode Levers arm system 310B Aurora Scientific Inc, Aurora Ontario Canada). The calcaneus and the tibia were perforated. The foot was mounted in such a way that it was possible to rotate the ankle to set positions, while strain was transferred through the Achilles tendon to the calcaneus. The ATF-sensor was mounted approximately at the height of the medial malleolus. The force transferred to the Achilles tendon was set to follow an approximation of the force profile during walking and scaled appropriately. **b** A qualitative comparison of the ATF-sensor output (thin black) and the force measurement (thick red) of a typical step cycle. **c** Different ankle rotations tested for the same force levels show that the ATF-sensor is also sensitive to the rotation of the ankle. **d** Correlation of ATF-sensor output normalised to 85° ankle rotation and force for the data in **c**.

7.4 *Ultrasound*

Ultrasound imaging of muscle fascicle movement during dynamic movement is an emerging field. It has been used to characterise fascicle movement during various types of highly dynamic tasks as drop jumping (Ishikawa and Komi 2004), running and walking (e.g. Lichtwark and Wilson 2006; Ishikawa *et al.* 2007). In ultrasound acquisition there is a positive correlation between the amount of noise in the image and the frequency of acquisition. As the noise level is increased the analysis of the final video becomes more difficult and prone to error, while if the acquisition frequency is decreased short lasting events could be missed and the error in onsets estimation of events would increase. We chose to use a relatively high acquisition frequency for the investigations, 150Hz for the within step modulation and 200Hz for muscle tendon unloading. Potentially a lower frequency could have been used for fascicle characterisation for the within step modulation, but initially it was thought that the timing of the minimum fascicle length could be important and hence the time resolution was kept high.

Manual tracking of the muscle fascicle in ultrasonographic video is an extremely time consuming process involving placing several markers on all the analysed frames. However, this is to date the most reliable method for analysing the noise prone high-frame rate ultrasound video. Some automatic tracking methods have been used to track length changes of tendinous tissues (Magnusson *et al.* 2003) and the contractile part of muscles (Loram *et al.* 2006). Unfortunately, these methods are not suitable for the current investigation in fascicle length.

To increase the accuracy of our measures, the same fascicle was analysed in three trials per condition and per muscle. The reliability of the current ultrasound method was determined by calculating the coefficient of variation between the three trials for each muscle and condition. The mean coefficient of variation was within previous reported values of <6% (Kurokawa *et al.* 2001; Ishikawa *et al.* 2003) and hence the reliability was deemed satisfactory.

8 Discussion

The objective of this PhD project was twofold; namely to investigate which proprioceptive pathways contribute to the locomotor activity of the soleus muscle in human walking and to study afferent pathways that shape the locomotor output of the plantar flexor muscles during over-ground walking. These questions were addressed by two separate setups. The first setup artificially unloaded the plantar flexor muscles in mid and late stance and the second setup mimicked walking on slightly uneven ground. The inherent restriction to indirect measures when studying human subjects does however limit the interpretation of the results, but in brief the combined conclusion of study II-IV was that the contribution of the soleus locomotor activity was load dependent. This load dependency was most likely mediated through the force sensitive group Ib afferent pathway (see discussion section 8.1 below). Furthermore, sensory afferent feedback was found to modulate the ongoing locomotor output of the triceps surae in a functionally sound manner, study I, IV. This modulation was most likely the result of excitatory feedback from group II and/or group Ib (see discussion section 8.2). Hence, proprioceptive or sensory feedback may enhance and adapt the locomotor output to fit the walking environment. As the investigations composing this dissertation have selectively investigated one of these two forms of feedback and the discussion of these studies have been divided accordingly.

8.1 Afferent pathways contributing to the locomotor activity

As highlighted in the introduction, it is better to investigate the contribution of afferent feedback by transiently decreasing rather than enhancing the afferent drive (Sinkjaer *et al.* 2000; Nielsen and Sinkjaer 2002; Donelan and Pearson 2004b; Donelan *et al.* 2009). By transiently removing or decreasing the sensory drive from a specific pathway, the importance of this pathway can be measured by the decrease in locomotor activity. However, with this approach it is difficult to determine whether the afferent drive is gating other input to the motoneurons or in itself part of a continuous feedback loop, i.e. also modulating the locomotor output. In study II and III and IV the decrease in afferent drive was accomplished by either rapidly decreasing the ground reaction force in the single support phase of stance or by inducing a rapid plantar flexion perturbation. These perturbations would decrease the sensory feedback and consequently a substantial depression in the soleus locomotor activity was found. Several afferent and central pathways may be involved in the generation of this response.

Descending or central influence is not likely to have major influence on the initial phase of the unload response. Firstly, the latency of the depression in locomotor activity following the unloading perturbation is short (~50ms and ~60ms for over-ground and treadmill respectively). The difference between the two situations is likely to stem from two sources of error in the onset latency estimate used in the

treadmill experiments. First, the onset of the ankle plantar flexion perturbation is for some subjects difficult to pinpoint on the ankle trace. Second, the transfer of the movement of the functional joint to the ankle joint is dependent on how tightly the joint is fitted to the ankle, while in the over-ground situation the ground reaction force reduction directly influences the reaction moment on the foot. Nevertheless, both of these onset latencies are relatively small when comparing to the short latency reflex which has an onset latency on approximately 40ms (Sinkjaer *et al.* 1996). However, these latencies do not per se exclude the supra spinal influence. The drop of the platform unloading the plantar flexors is a whole body perturbation. The drastic decrease in ground reaction force is inevitably transferred to an acceleration of the head in the vertical direction. Hence, there could be a vestibulospinal contribution to the measured response (Fitzpatrick *et al.* 1994). As it turns out, when investigating the acceleration of the head, the likelihood of a vestibulospinal influence is small as the acceleration of the head is significantly delayed with respect to the change in ground reaction force, see study II.

Another pathway that could contribute to the depression in the soleus is feedback from the cutaneous afferents. Cutaneous afferents from the sole of the foot have been shown to play an important role in regulating locomotor activity during walking (for review see: Zehr and Stein 1999; Duysens *et al.* 2000). For example, cutaneous non-noxious stimulation of the sural nerve during swing elicits a response that moves the limb away from the stimuli or obstacle (Zehr *et al.* 1998). This is also the case for stimuli during late stance which produce functionally relevant movement away from the stimuli (Zehr *et al.* 1998). Zehr *et al.* suggested that the response in stance may prevent destabilising of the limb in stance by altering the ankle trajectory when stepping on slanted support surface. It is clear that transient cutaneous activation does elicit motor responses during human walking. Nevertheless, the rapid decrease in ground reaction force or the rapid plantar flexion perturbations would invariantly impose changes to the load distribution of the foot. This change in pressure distribution would most likely also affect both cutaneous and proprioceptive afferent firing rates from the foot and ankle. These changes in sensory drive from the foot and ankle could potentially depress the soleus activity as reported by Shoji *et al.* (2005) after plantar nerve stimulation. Though, the reported inhibition from the plantar nerve stimulation has approximately the same latency, ~50ms, the inhibitory drive on the soleus was shown to decrease by loading. Furthermore, when depressing sensory afferents from the foot and ankle using local anaesthesia of all nerves supplying the foot and ankle, no significant effect was found on the depression in soleus activity after rapid plantar flexion perturbations (Grey *et al.* 2004). Therefore, it is unlikely that cutaneous afferents would have a significant impact on the soleus depression after rapid decrease of the ground reaction force or when the ankle was rapidly plantar flexed.

It was already suggested by Sinkjaer *et al.* (2000) that it is the cessation of group II and/or group Ib afferents that contribute to unload response. They favoured group II afferents and the group II pathway was also suggested by Mazzaro *et al.* (2005; 2006) to be involved in modulating the locomotor activity. In contrast, the studies II–IV presented in this dissertation add to the body of evidence that favour the group Ib pathway. It should first be recognised that all investigations so far supply indirect evidence for one or the other pathway as neither a complete mapping of the spinal network nor direct recording from afferents during dynamic movements is possible with current techniques. Furthermore, there is an inherent difficulty in distinguishing between force and length sensitive afferents as force and length changes are mechanically coupled. This issue was approached by two methods used in the over-ground investigations, namely by constricting the ankle movement using of an ankle foot orthotic and by measuring the muscle fascicle movement rather than the muscle-tendon complex. By minimising the ankle movement, the ankle plantar flexion as a result of the reduction in ground reaction force was reduced by approximately 30%. This reduction in plantar flexion did not result in a significantly reduced response. It should be recognised that it is possible that the gain of the length sensitive group II afferent feedback could have been increased to compensate for the limited range of motion as the subjects were given ample time to adapt to the limited range of motion. However, the muscle activity was reduced when the subjects walked with the ankle foot orthotic. If the group II sensitivity was increased this would imply a decoupling of alpha-gamma coactivation and a significant increase in static gamma drive. Though possible and indications of differentiated alpha and gamma activation has recently been reported in man (Ribot-Ciscar *et al.* 2009), I believe it is more likely that the cessation of force sensitive afferents contributes to the unload response over-ground. Ultrasound characterisation of the muscle fascicle length after the drop of the platform support this as the shortening was significantly delayed with respect to the reduction in ground reaction forces, see study IV. This delay in the muscle fascicle shortening would leave very limited time for the group II afferent drive to integrate at the spinal level and contribute to the response. The second method used was to transiently change the body weight support while the subject walked on a treadmill. Unload responses were presented the second step after the change in body weight support, and it was found that the unload response was decreased when the load on the supporting limb was decreased. This finding parallels the correlation between the magnitude of the unload response and the force reduction in the Achilles tendon imposed by the plantar flexion perturbations (Grey *et al.* 2007). Also, in study III the Tizanidine depression of the group II afferent pathway did not significantly change the unload response. Though this further emphasises the likelihood that other pathways than the group II afferent pathway contribute to the unload response, it should be noted that 1) the power of the investigation does not rule out small changes in the unload response as a result of the depression of the group II pathway, and 2) the Tizanidine induced depression is not transiently induced,

and hence there is a possibility that the influence of the non-affected pathways was increased.

To recapitulate, it is hence likely that force sensitive group Ib afferents do contribute to the unload response in human walking both over-ground and when imposed plantar flexion perturbations are presented on the treadmill. However, the contribution from group II afferents and other afferents is not excluded and they may also contribute to the locomotor activity but to a lesser extent and/or with longer delays.

What can the unload response tell us about the physiology of afferent integration in unperturbed human walking? The rationale behind the unload response is that by breaking the feedback loop the difference in activity between the unperturbed and perturbed steps is a measure of the feedback loop's contribution to the muscle activity. In other words transiently removing afferent drive should reveal its existence on the locomotor activity as a decrease in activity. It should be stressed here that when discussing the unload response and the afferents contributing to the response, this is in fact the lack of this afferent drive that produces the response, a dis-facilitation of the motoneuron. This should be distinguished from active inhibitory drive through for example reciprocal inhibition from the stretch of antagonist muscles. Moreover, the implication of the magnitude of the unload response should be carefully discussed as there are nonlinearities in the integration of afferent drive. For example, for a motoneuron receiving just enough excitatory input to fire, removal of any drive will keep the motoneuron hyperpolarised below the firing threshold. Hence, it is impossible to infer from the unload response a precise amount of locomotor activity generated through afferent feedback as the excitability of the spinal motoneuronal pool is unknown. Nevertheless, the unload response gives a clear indication of the importance of the afferent drive and whether or not a certain pathway is involved in raising the excitability of the motor pool. Although it should be recognised that even without the support of afferent drive to the motoneurons, as for the deafferented subject studied in study I, the muscle can be consciously activated by more central structures to perform the contractions that are needed to walk.

It is also interesting to note the difference between the control of walking in cats and in humans. The classical foot-in-hole experiments, where the cat unexpectedly stepped into a hole with a hind limb, showed an initial depression in the plantar flexor muscles (Gorassini *et al.* 1994). This loss of plantar flexor activity could stem from the loss in sensory feedback from the limb. However, when a similar experiment was performed on human subjects the plantar flexor activity was increased rather than decreased when the subject unexpectedly stepped into a hole (van der Linden *et al.* 2007). This difference in response likely has its origin in the higher need for postural adjustments in bipedal as compared to quadruped walking. The latency for this response was measured in the gastrocnemius medialis muscle to 47ms (van der Linden *et al.* 2007). On the other hand, when the limbs are loaded at the time when the unloading perturbation is presented,

both in human and in cat the plantar flexor muscles are decreased (in human: Sinkjaer *et al.* 2000; af Klint *et al.* 2009; in cat: Donelan and Pearson 2004b; Donelan *et al.* 2009). The extent of the unload response is greater for the localised plantar flexion perturbations (study III) in human as compared to the over-ground induced unloading (study II, IV). The shorter extent of the response to the unload in the over-ground situation could potentially be due to a compensatory postural reflex as found by van der Liden *et al.* (2007) raising the muscle activity to pre-contract the plantar flexor muscles for the inevitable landing.

8.2 Afferent pathways modulating the locomotor activity

When walking over slightly uneven ground the plantar flexor muscles activation is adapted to the inclination of the surface. 5 levels of rotation in the parasagittal plane (-3° , -2° , 0° , $+2^\circ$, $+3^\circ$) were tested in Study I and three levels in study IV. The inclinations and declinations of the supporting surface generated, respectively, within-step enhancements or reductions in the locomotor activities of the triceps surae muscles. These enhancements and reductions in locomotor EMG were the result of an overall activity change in the muscles and not due to transient effect at heel strike. Therefore, the functional significance of this ongoing within-step modulation of the plantar flexor activity may well be to stabilise the forward propulsion and/or increase the stability of gait.

The slightly uneven surface used during the current investigations is a significantly different perturbation than the imposed ankle dorsiflexion velocity enhancements and reductions previously studied by Mazzaro *et al.* (2005; 2006). The slightly uneven surface does not impose movements of the ankle, but mimics the natural variation in ground surface that we meet when walking outside. The inclination of the supporting surface could only be changed for the step on the platform and so the subjects were faced with an uneven surface for one step every 10m. On the other hand, imposing small amplitude slow velocity dorsiflexion enhancements or reductions change the dynamics of the ankle movement during the step. These perturbations are difficult to relate to something that would normally be faced during walking. Potentially the perturbations could be seen as walking on a surface that gives away (reduced dorsiflexion velocity) and a situation where the support surface propels the subject forward ever so slightly (increased dorsiflexion velocity).

Nevertheless, the imposed dorsiflexion velocity enhancements studied by Mazzaro *et al.* (2005; 2006) increased the soleus muscle activity in stance. This increase was found to be velocity dependent and was decreased by ischemic block of the largest group I afferents (Mazzaro *et al.* 2005). On the other hand the dorsiflexion velocity reductions were not changed by the ischemic block. Therefore, Mazzaro *et al.* (2005) concluded that increases in muscle activity was influenced by feedback from group Ia, while other afferents attributed to the reductions in locomotor activity. This conclusion is contradictory to what was found in the study I and study IV. In study I the muscle-tendon lengthening velocity was

inversely correlated to the increase in triceps surae activity. Furthermore, in study **IV** the muscle fascicles did not show any significant change in lengthening velocity while the locomotor activity was increased when stepping on an inclined surface. These results indicate that it is unlikely that group Ia contributes significantly to increasing locomotor activity in over-ground unrestrained human walking. The discrepancy could potentially be attributed to the imposed enhancement of the dorsiflexion velocity elicits changes in the firing rates of the group Ia velocity sensitive afferent that are not encountered normally during walking. The imposed changes in dorsiflexion velocity were small and burst-like stretch reflex activity in the muscle activity avoided. However, without the direct measurement of afferent activity it is still possible that the increased dorsiflexion velocities change the temporal dispersion of some of the group Ia afferents. This could make their excitatory drive on the motoneuron pass the presynaptic inhibition and thereby increasing the muscle units' activity even without detectable bursting activity. The lack of bursting muscle activity does not rule out changes in the temporal dispersion of the group Ia, and as changes in dorsiflexion velocity is hardly ever encountered in unrestrained walking this cannot constitute a model for afferent feedback in normal over-ground walking.

Both feedback from group II length sensitive afferents as well as group Ib force sensitive afferents has been suggested to modulate locomotor activity in human. The estimate of the Achilles tendon force was increased with stepping on the inclined surface and decreased when stepping on the declined surface. Similarly, the length of the Achilles tendon was increased for the positive inclinations indicating higher strain on the tendon. Consequently, we can assume that the stepping on the slightly inclined and declined supporting surface, respectively, enhanced or reduced the tension on the Achilles tendon. The higher tension is assumed to raise group Ib afferents firing rates (Houk and Henneman 1967). However, concomitantly both the muscle-tendon length and the muscle fascicle length were also increased and decreased with stepping on the inclined and declined surface. These length changes are assumed to mostly affect the firing rates of group II afferents, but also group Ia afferents are to some extent length sensitive (Brown *et al.* 1965; Matthews and Stein 1969a). Consequently, the contribution from positive length and force feedback to the modulation of the locomotor activity could not be separated using the current techniques.

Experiments on cats have shown that positive force feedback influences the locomotor activity during walking (Donelan *et al.* 2009). While there is no direct evidence of positive length feedback (Donelan and Pearson 2004a; Donelan and Pearson 2004b; Donelan *et al.* 2009). At least partly, the potential functionally different role of group II afferents in human and cat could be attributed to the difference in muscle lengthening. In humans the soleus is undergoing a larger eccentric contraction during stance, giving the length signal a potentially more important role in the feedback (Nielsen 2003). On the other hand, the role of force feedback is still a matter of debate in human walking. The Ib inhibition

from gastrocnemius medialis to the soleus muscle was investigated through paired electrical nerve stimulation (Faist *et al.* 2006). They found that the inhibition was reduced or disappeared as the leg was loaded and during the stance phase of “reduced” gait the inhibitory connection was in most cases replaced by an excitatory Ib feedback. This indicates that heterogenic positive Ib feedback likely exists in the human walking. However, using this technique the strength of autogenic feedback in the soleus muscle could not be investigated, which remains unclear. Nevertheless, from a control point of view, it is more likely that both length and force information is used to control the muscle activity during walking. Redundancy of information not only makes the system less sensitive to disruptions of one of the modalities. It is also likely that the added information from using the two modalities would improve the accuracy of the internal model and therefore improve the control.

8.3 *Final comments*

All through this dissertation the afferent modulation and afferent contribution to the locomotor activity have been separately investigated and discussed. This initial separation stems historically from the conclusions that could be drawn by the unload response. However, as discussed above the same afferents that contribute may also be involved in the modulation of the locomotor activity. This makes it likely that the same afferent pathways also are involved for both the contribution and modulation. This was also highlighted by the modulation of the unload response with both body weight support (study III) and with inclined and declined walking (Grey *et al.* 2007). Though such a modulation was not investigated for the over-ground unload response, it is likely that similar pathways are involved for the over-ground induced response as for plantar flexion induced response. Hence, the over-ground response would also be modulated by the group Ib and/or group II afferents.

Group Ib afferent drive has an excitatory feedback to the motoneurons during the stance phase of walking in cats (Pearson and Collins 1993; Donelan *et al.* 2009). This continuous afferent feedback loop both modulates and supports the central drive to the locomotor activity. The group II afferents have similar connectivity to the motoneurons, but as previously mentioned the ankle extensors in the cat only go through a small lengthening contraction limiting the usability of length feedback. Furthermore, some 60% of the last order intermediate zone/ventral group II interneurons receive input not solely from group II afferents but also from both group Ia and/or group Ib in cats (for review: Jankowska 1992). This would imply that the excitatory group II pathway may be influenced by both the velocity sensitive group Ia drive and the force sensitive group Ib drive. If the connectivity to the interneurons in humans is similarly distributed, this could also account for the difference in MLR seen after changes in body weight support in study III. In the current studies (I, III, IV) the contribution and modulation of locomotor activity was seen to be dependent on mostly group Ib and group II

afferent pathways. Given the interconnectivity of the two pathways in the spinal cord it is therefore likely that no one pathway may be completely separated out from the others.

From a control point of view it may also be advantageous to use at least two modalities of afferent information in the control loop. Forces applied to a body integrated over time results in movement. If all forces are known and the initial condition, i.e. mass, moment of inertia, position and velocity of all the bodies segments, the movement and resulting position and orientation of the body may be calculated. However, there exists no ideal sensors and the internal representation of the segments mass and moment of inertia will change over time or with wearing clothes. By fusing information from both force and length sensors, systematic integration errors, from the integration of forces, may be limited as it has a boundary in the separate length measurements. Furthermore, from a redundancy perspective it would be beneficial with more than one afferent modality, where the loss of or depression of a certain pathway may be compensated by an increase in the loop gain of the remaining pathways. Therefore, it is important not to overemphasise the results of the Tizanidine induced group II afferent pathway depression in study III. As the Tizanidine depression was hardly transiently induced, the potential adaptation of the spinal interneurons may have increased the gain of the non-depressed pathways leaving the unload response unaltered. However, the latency estimate of the ultrasound measured muscle fascicles trajectories showed for the over-ground unload response that group II are most likely not involved.

During the last 20 years, spinal segmental feedback from spindle afferents has been thought to contribute to human walking. H-reflexes and stretch reflexes were analysed under the assumption that the consistent and well defined reflexes indicate how the group Ia activity integrates with central locomotor activity to generate the human locomotor activity (Gollhofer *et al.* 1984; Capaday and Stein 1986; Crenna and Frigo 1987). Group Ib feedback was largely ignored as it has an inhibitory projection to the motoneurons during standing, which is now known to be depressed and even excitatory during walking (Stephens and Yang 1996; Faist *et al.* 2006). As the tools of these investigations improved more information was gathered on the stretch reflex (Yang *et al.* 1991; Sinkjaer *et al.* 1996; Grey *et al.* 2001). Similarly in the cat, the spindle afferents were thought to play a crucial role in the generation of locomotor activity as the muscle activity was significantly decreased by nerve block of the fusimotor axon (Severin 1970). However, this view diversified in the recent years as firstly the inhibitory disynaptic group Ib projections were shown to switch to excitatory feedback during the stance phase of walking (Conway *et al.* 1987; Pearson and Collins 1993; Whelan *et al.* 1995b). Secondly, the importance of this positive force feedback loop was clearly shown through careful experimental designs, separating force changes from length changes of the ankle extensor muscles, (Donelan and Pearson 2004a; Donelan and Pearson 2004b; Donelan *et al.* 2009). The current

investigations are in effect showing, yet indirectly, that the positive force feedback present in the walking cat is also present in the ankle extensors of human walking. Functionally, such a load and/or force feedback contributing to the muscle activity could automatically adjust muscle activity to compensate for higher loads faced when wearing a rucksack or walking on a slightly uphill slope. Potentially this feedback also aides in maintaining the body's projected centre of mass within the base of support (Dietz 1992) or to simply maintain the propulsive forces. Both of these control goals are important to keep a stable gait, but extending the current studies on the ankle joint to the multi-segmental body is difficult. Nevertheless, as the ankle extensor activity in stance phase is crucial for the propulsive forces, at least for the late stance, feedback from force sensors have functionally a potentially very important role in the control of human bipedal walking.

9 References

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